(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 077 218 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
21.02.2001 Bulletin 2001/08

(51) Int Cl.[7]: C07K 7/56

(21) Application number: 00830535.1

(22) Date of filing: 27.07.2000

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 04.08.1999 US 366198

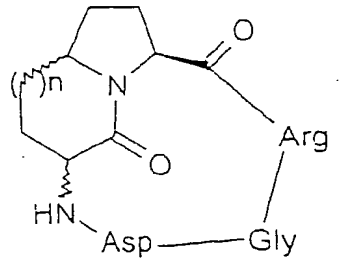
(71) Applicant: SIGMA-TAU Industrie Farmaceutiche Riunite S.p.A.
00144 Roma (IT)

(72) Inventors:
- Scolastico, Carlo
  20100 Milano Due (Segrate Milano) (IT)
- Giannini, Giuseppe
  00040 Pomezia, Rome (IT)

(74) Representative: Spadaro, Marco
Sigma-Tau
Industrie Farmaceutiche Riunite SpA,
47, Viale Shakespeare
00144 Rome (IT)

(54) Peptido-mimetic compounds containing RGD sequence useful as integrin inhibitors

(57) The present invention discloses compounds of formula (I)



(I)

wherein n is the number 0, 1 or 2. There are also disclosed processes for the preparation of said compounds, together with methods for treating pathologies related to an altered $\alpha_v\beta_3$ integrin-mediated cell attachment, in particular wherein the inhibition of angiogenesis is desired, for example in tumors, also associated with metastasis.

Derivati senza sostituzione in posizione 3 (R_3=H)

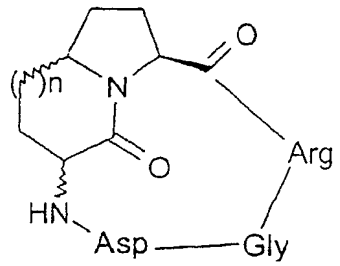
EP 1 077 218 A2

[0036] WO98/56407 and WO98/56408 disclose fibronectin antagonists as therapeutic agents and broad-spectrum enhancers of antibiotic therapy. Said fibronectin antagonists bind to a $\alpha_5\beta_1$ integrin to the purpose to prevent intracellular invasion by microbial pathogens. Some of these inhibitors are linear or cyclic peptides containing the RGD structure or antibodies. Integrin antagonists are specifically disclosed for their selectivity against $\alpha_5\beta_1$ integrin. The best of them proved to be (S)-2-[2,4,6-trimethylphenyl)sulfonyl]amino-3-[[7benzyloxycarbonyl-8-(2-pyridinylaminomethyl)-1-oxa-2,7-diazaspiro-[4,4]-non-2-en-3-yl]carbonylamino]propionic acid.

[0037] US 5,773,412 discloses a method for altering αvβ3 integrin receptor-mediated binding of a cell to a matrix, said cell being an endothelial or smooth muscle cell, by contacting said cell with a RGD-containing cyclic peptide. Also disclosed there is a method for inhibiting angiogenesis by using this cyclic peptide. The cyclic peptide disclosed in US 5,773,412 contains at least 6 amino acids and the RGD sequence is flanked, on the D-side, by a first amino acid which can provide a hydrogen bond interaction with an integrin receptor (Asn, Ser or Thr) and a second amino acid, that has the characteristics of hydrophobicity or conformational constraint (Tic, i.e. 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Pro, Phe or Ile). A selection of these peptides are taught as useful for altering the binding of osteoclasts to a matrix such as bone or for selectively altering integrin receptor binding. It has now been found that cyclic pseudopeptides having an RGD mimetic structure characterized by an azabicycloalkane structure are endowed with selective inhibition of αvβ3 integrin-mediated cell attachment. This activity makes them useful as therapeutical agents, in particular for treating pathologies due to an altered angiogenesis, for example tumors.

Abstract of the invention

[0038] It is an object of the present invention, compounds of formula (I)



(I)

wherein n is the number 0, 1 or 2,

Arg is the amino acid L-Arginine, Gly is the amino acid Glycine and Asp is the amino acid L-Aspartic acid, and the pharmaceutically acceptable salts thereof, their racemates, single enantiomers and stereoisomers.

[0039] The compounds of formula (I) are selective inhibitors of $\alpha_v\beta_3$ receptor. Accordingly, they are useful for treating all those pathologies due to an altered $\alpha_v\beta_3$ integrin-mediated cell attachment; for example, retinopathies, acute renal failure, osteoporosis, tumors, also associated with metastasis. The compounds of the present invention can be considered as antiangiogenesis agents, in particular for the treatment of tumors, comprising tumors associated with metastasis.

[0040] Other objects of the present invention are processes for the preparation of the compounds of formula (I).

[0041] A further object of the present invention is a method for treating a subject, whether human or animal, suffering of a tumor, by inducing an inhibition of angiogenesis, in particular for inhibiting or reducing or blocking metastatic proliferation, with the administration of a therapeutic or preventive dose of at least a compound of formula (I). Also objects of the present invention are: a method for selectively inhibiting $\alpha_v\beta_3$ integrin-mediated cell attachment to an RGD-containing ligand, comprising contacting said ligand with an effective amount of a compound of formula (I); a method for treating a subject suffering from a pathology related to an altered $\alpha_v\beta_3$ integrin-mediated cell attachment comprising administering to said subject a compound of formula (I); said pathologies being for example retinopathy, acute renal failure, osteoporosis.

[0042] From the industrial application point of view, the present invention also comprises pharmaceutical compositions comprising an effective dose of at least a compound of formula (I) in admixture with pharmaceutically acceptable vehicles and/or excipients.

[0043] The present invention shall be disclosed in detail in the foregoing also by means of examples and figures, wherein, in the figures:

Figure 1 represents, in an exemplary way, the general synthesis of the lactams;
Figure 2 represents a preferred embodiment of the synthesis of 6,5-fused "cis" lactams;
Figure 3 represents a preferred embodiment of stereoselective hydrogenation with chiral phosphine-Rh catalyst;
Figure 4 represents a preferred embodiment of the synthesis of 7,5-fused "cis" lactams;
Figure 5 represents a preferred embodiment of the synthesis of 5,5-fused "cis" lactams;
Figure 6 represents another preferred embodiment of the synthesis of 5,5-fused "cis" lactams;
Figure 7 represents a preferred embodiment of the synthesis of 6,5-fused "trans" lactams;
Figure 8 represents a preferred embodiment of the synthesis of 7,5-fused "trans" lactams.
Figure 9 represents a preferred embodiment of bicyclic lactam templates Fmoc-protected;
Figure 10 represents a preferred embodiment of linear pseudopeptides tBu-, Pmc-protected;
Figure 11 represents a preferred embodiment of protected cyclic pseudopeptides;
Figure 12 represents a preferred embodiment of RGD cyclic pseudopeptides;

Detailed description of the invention

[0044] In its broadest aspects, the present invention relates to compounds of the above formula (I).

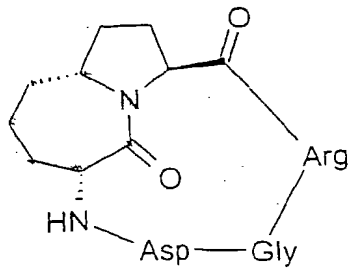
[0045] The compounds of formula (I) are peptido-mimetics containing an RGD sequence. Said compounds can be seen as formed by an azabicycloalkane scaffold and an RGD sequence.

[0046] For sake of clarity, in formula (I), there is a variable part, given by the different values of n, and a fixed part, given by the RGD sequence. When n is 0, the scaffold is referred to as 5,5 azabicycloalkane, when n is 1, the scaffold is referred to as 6,5 azabicycloalkane and when n is 2, the scaffold is referred to as 7,5 azabicycloalkane. The bonds written in formula (I) as a wavy line represents a stereo bond, which can be either above the plane of the page (thick bond) either below the plane of the page (thin bond). The compounds of formula (I) can exist in different stereoisomers, according to the orientation of the wavy bond.

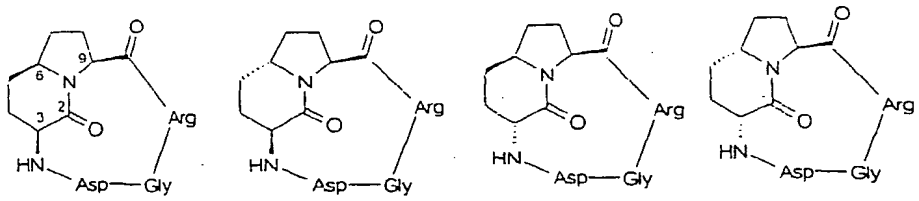
[0047] A first class of preferred compounds of formula (I) are 7,5 azabicycloalkane, in particular those having trans configuration as to the positions 7 and 10 and (R) configuration as to the carbon atom at position 3.

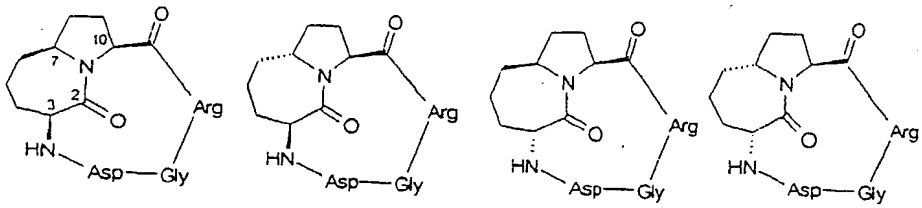
[0048] A second class of preferred compounds of formula (I) are 6,5 azabicycloalkane, in particular those having trans configuration as to the positions 6 and 9 and (S) configuration as to the carbon atom at position 3.

[0049] A particularly preferred compound is the one of the following formula (also named as ST 1646).



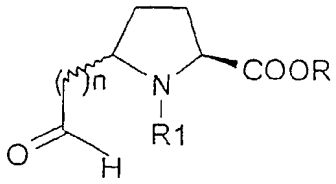
[0050] In the following table there are represented the preferred compounds of formula (I):





[0051] Within the boundaries of the present invention, there is disclosed a process for the preparation of the compounds of formula (I), comprising the following steps:

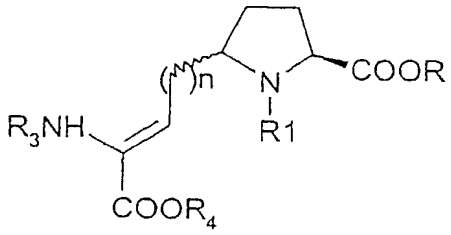
a) Horner-Emmons olefination of a compound of formula (II)



(II)

wherein

R is a lower alkyl residue;
R_1 is a suitable nitrogen protecting group, to give a compound of formula (III);



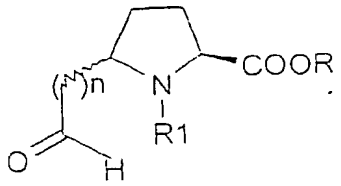
(III)

wherein R_3 is a suitable nitrogen protecting group, R_4 is a lower alkyl residue;

b) hydrogenation of said compound of formula (III) and cyclisation; and, if desired
c) separation of the stereoisomeric mixture;
d) building of the RGD cyclic sequence, and if desired
e) separation of the stereoisomeric mixture.

[0052] A process for the stereoselective synthesis of the compounds of formula (I), comprises the following steps:

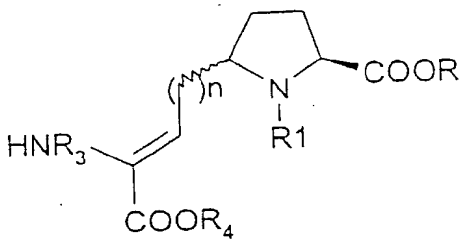
a) Horner-Emmons olefination of a compound of formula (II)



(II)

wherein

R is a lower alkyl residue;
R_1 is a suitable nitrogen protecting group, to give a compound of formula (III);



(III)

wherein R_3 is a suitable nitrogen protecting group, R_4 is a lower alkyl residue;

b) hydrogenation of said compound of formula (III) by chiral phosphine-Rh catalysed hydrogenation and cyclisation; and, if desired
c) separation of the stereoisomeric mixture;
d) building of the RGD cyclic sequence and if desired
e) separation of the stereoisomeric mixture.

[0053] As lower alkyl residue it is normally understood a C_1-C_4 alkyl, for example, methyl, ethyl, propyl, butyl and all the possible isomers, but also higher alkyls are suitable, provided their compatibility with reaction conditions. As suitable nitrogen protecting groups, the skilled person is able to select, according the general common knowledge, the suitable protecting group, as it will appear from the following examples , but also in the available technical literature and commercial catalogues.

[0054] Also disclosed are pharmaceutical compositions comprising a therapeutically or preventive effective dose of at least a compound of formula (I) in admixture with pharmaceutically acceptable vehicles and/or excipients.

[0055] In its broadest aspect, the present invention advantageously teaches a method for selectively inhibiting $\alpha_v\beta_3$ integrin-mediated cell attachment to an RGD-containing ligand, comprising contacting said ligand with an effective amount of a compound of formula (I), a method for treating a subject suffering from altered angiogenesis, comprising administering to said subject a compound of formula (I), a method for the treatment of tumors in a subject comprising administering to said subject a compound of formula (I), optionally in combination with other active ingredients, in particular other antitumour agents.

[0056] The present invention shall be described in detail also by means of examples and figures, wherein,

Best mode for carrying out the invention

[0057] The synthesis of so-called peptido-mimetics molecules has been a very active and productive field of research in drug design (J. Gante, Angew. Chem., Int. Ed. Engl. 1994, 33, 1699. - G.L. Olson, et al.: J. Med. Chem. 1993, 36, 3039. - D.C. Horwell, Bioorg.Med. Chem. Lett. 1993, 3, 797. - A. Giannis et al.: Angew. Chem., Int. Ed. Engl. 1993, 32, 1244. - B.A. Morgan: Annu. Rep. Med. Chem. 1989, 24, 243). The expectation is that these molecules will have the same biological effects as natural peptides, but at the same time, will be metabolically more stable. Of particular

interest has been the replacement of reverse-turn dipeptide motifs with constrained molecules that reproduce their conformational features (ibid; M. Kahn, Ed., Peptide Secondary Structure Mimetics. Tetrahedron Symposia-in-Print No. 50 1993, 49, 3433-3689 and references therein). This goal has been frequently achieved using the azaoxobicyclo [X.Y.O]alkane skeleton and/or heteroatom analogues. This has created a demand for efficient synthetic approaches toward such molecules, and many methods have been introduced and recently reviewed (S. Hanessian et al: Tetrahedron 1997, 38, 12789-12854). One particularly effective and versatile route has been developed by Lubell et al. and employed for the preparation of enantiopure indolizidinone-type 6,5-fused bicyclic lactams (H.-G. Lombartet al.: J. Org. Chem. 1996, 61, 9437-9446. - F. Polyak et al.: J. Org. Chem. 1998, 63, 5937-5949 and references therein for the syntheses of azabicycloalkane amino acids -- F. Gosselin et al.: J. Org. Chem. 1998, 63, 7463-7471). Several procedures are also available for the synthesis of 7,5-fused bicyclic lactams, the majority of which require relatively long synthetic sequences. On the contrary, there is not many published protocol that allow the synthesis of 5,5-fused bicyclic lactams.

[0058] According to the present invention, the beta-turn portion of the cyclic peptide consists in an azabicycloalkane amino acid scaffold, selected from a 5,5-, 6,5- or 7,5-fused bicyclic lactams. Several 6,5- and 7,5-fused 1-aza-2-oxabicyclo[X.3.0]alkane amino acids have been synthesised, using radical (L. Colombo et al.: Tetrahedron Lett. 1995, 36, 625-628. - L. Colombo et al.: Gazz. Chim. It. 1996, 126, 543-554) or ionic reactions (L. Colombo et al. Tetrahedron 1998, 54, 5325-5336). These structures can be regarded as conformationally restricted substitutes for Ala-Pro and Phe-Pro dipeptide units, and, if their conformations meet certain criteria, they can be used to replace the central (i+ 1 and i+2) residues of β-turns.

[0059] The present invention provides an improved reaction sequence, amenable to large scale preparation, and allowing the synthesis of different bicyclic lactams from common intermediates, as described in the appended Figure 1.

[0060] Starting from 5-allyl/formyl prolines **13-18**, a Z-selective Horner-Emmons olefination followed by double bond reduction has been used to build the second ring. The starting aldehydes have been stereoselectively synthesised by modifications of known procedures (vide infra). Stereorandom double bond reduction can be performed using H_2/Pd to yield, after cyclisation, mixtures of easily separable epimers. Stereoselective hydrogenation is studied for the synthesis of 6,5-fused lactams, and achieved with d.e. 80% using Rh-chiral phosphine catalysts. Structural diversity, in terms of ring size and stereochemistry of the azabicycloalkane fragment, is provided by the new strategy, and access to the less common 5,5-fused bicyclic scaffold is also secured.

[0061] Examples of bicyclic dipeptide derivatives **1-12** are shown in Figure 2.

Synthesis of the fused bicyclic lactams 1-12

[0062] The synthesis of lactams 1-12 follows the common steps reported in Figure 1. Starting from the cis or trans 5-alkyl proline aldehydes 13-18, a Horner-Emmons olefination with the potassium enolate of (±)-Z-α-phosphonoglycine trimethyl ester (U. Schmidt, A. Lieberknecht, J. Wild, Synthesis 1984, 53-60) sets up the necessary carbon chain. Following protecting group manipulation (vide infra), reduction of the enamino acrylic acids and treatment with condensing agents gives the lactams of both the "cis" and "trans" series in good yields.

[0063] In all cases where stereoisomeric mixtures of lactams are formed, they can be easily separated by flash chromatography, and their configuration can be assigned with n.O.e. experiments.

[0064] The synthetic scheme is best illustrated by the synthesis of the 6,5-fused "cis"-lactams **2a** and **8a** (Figure 3). The necessary cis aldehyde **14** is obtained from the known cis 5-allyl-proline derivative **25** (M. V. Chiesa, L. Manzoni, C. Scolastico, Synlett 1996, 441-443) and reacted with the commercially available phosphonate **26** (U. Schmidt, A. Lieberknecht, J. Wild, Synthesis 1984, 53-60) to give **20** in 98% yield and 7:1 Z:E ratio.

[0065] Hydrogenation of **20** occurs initially at the enamino Cbz group, and thus results in a complex mixture of products. To circumvent this problem, the substrate is treated with Boc_2O to give **27** (98%). Reduction of **27** with H_2/Pd $(OH)_2$ followed by reflux in MeOH gives a 1:1 mixture of **8a** and **2a**, which are easily separated by flash-chromatography. From **14** the whole sequence requires only two chromatographic separations (purification of **20** and separation of **8a** from **2a**) and can easily be carried out in multigram scale.

[0066] The stereoselective preparation of the two epimers **8a** and **2a** (Figure 3) is carried out using chiral phosphine-Rh catalysed hydrogenation of the enamino acid **28**.

[0067] Chiral phosphine-Rh catalyst is well-known to represent a powerful and well-established way of access to naturally and non-naturally occurring amino acids and the catalytic asymmetric hydrogenation of dehydropeptides is the logical extension of this methodology to the preparation of biologically active chiral oligo- and polypeptides.

[0068] In asymmetric catalytic hydrogenations using chiral phosphine-Rh catalysts (Z) olefins usually gives the highest stereoisomeric purity of the products, but the most stringent requirement for the substrate remains the presence of an acetamido or an equivalent group on the double bond. (K.E. Koenig in Asymmetric Synthesis, J.D. Morrison Editor, Vol 5, Academic Press Inc. 1985, 71) The amide-type carbonyl is needed in order to allow two-point co-ordination of the substrate to the metal, which increases the sterical demand as it has been fully elucidated experimentally. (J.

Halpern, ibidem, 41) For applications to the synthesis of peptides protecting groups other than the acetamido, like Boc or Cbz should be used, thus permitting differential deprotection. However, very few examples of asymmetric catalytic hydrogenation are known in which these protecting groups are found on the enamino nitrogen: (B. Basu, S.K. Chattopadhyay, A. Ritzen, T. Frejd, Tetrahedron Asymmetry, 1997, 8, 1841) (S.D. Debenham, J.D. Debenham, M.J. Burk, E. J. Toone, J.Am.Chem.Soc. 1997, 119, 9897) more frequently Boc or Cbz protecting groups are present in different position of dehydropeptides being hydrogenated at the N-terminus. (A. Hammadi et al. Tetrahedron Lett. 1998, 39, 2955 - I. Ojima, Pure & Appl. Chem. 1984, 56, 99). For the catalytic asymmetric hydrogenation of **28** [Rh(Phosphine)(COD)]ClO_4 catalysts is used. The catalysts were prepared by displacing one cyclooctadiene ligand of [$Rh(COD)_2$]ClO_4 with the appropriate phosphine. The ligands investigated are (R)-Prophos 29 and (+) or (-) BitianP 30 and 31. BitianP is a chiral atropisomeric chelating phosphine belonging to a new class of ligands based on biheteroaromatic framework, which gives very high e.e.% in the asymmetric hydrogenation of olefins and ketones. (E. Cesarotti et al. J.Chem.Soc.Chem.Comm. 1995, 685 - Cesarotti et al. J.Org.Chem. 1996, 61, 6244).

[0069] The results of asymmetric hydrogenation are reported in the Table 1. The conversion is always quantitative but the highest stereodifferentiation is obtained with [Rh/(-)-BitianP] (entry 3). The results suggest that the newly created stereocentre is mainly determined by the catalyst, which overruns the effect of the stereocentre on the substrates (entry 2 and 3) The results also indicate that the Boc protecting group on the enamino nitrogen fulfils the requirements and allows the olefin to chelate to the catalyst.

Table 1

Asymmetric hydrogenation of 28			
Entry	Catalyst	**32/33**	d.e. %
1	Rh-**29**	86/14	72
2	Rh-**30**	13/87	74
3	Rh-**31**	90/10	80

- Reactions were carried out at R.T. for 24 h under 10 atm of H_2

[0070] Treatment of crude **32** and **33** with CH_2N_2, followed by hydrogenation and cyclisation under the usual conditions (H_2/Pd-C followed by reflux in MeOH) allows a stereoselective route to lactams **8a** and **2a**.

[0071] All the remaining lactams **1-12** can be synthesised following essentially the same sequence described above. Thus, the 7,5-fused lactams **3a** and **9a** (Figure 4) can be made starting from the cis aldehyde **15**, easily prepared from the cis 5-allyl proline **25**. (M. V. Chiesa, L. Manzoni, C. Scolastico, Synlett 1996, 441-443) Horner-Emmons reaction of **15** with **26** gives a 6:1 Z:E mixture of enamino acrylates. After N-protection they are reduced with H_2/Pd-C. The thermic cyclisation of methyl ester **34** can be carried out in a suitable solvent, for example xylene. Better results are obtained upon ester hydrolysis followed by EDC/HOBT promoted lactam formation to give **3a** and **9a**, which are easily separable by flash chromatography (51% overall yield from **25**).

[0072] The starting material for the synthesis of the 5,5-fused "cis" lactams (Figure 5) is alcohol **36**. Oxidation and Horner-Emmons reaction with 26 followed by N-Boc protection gives **37** as a 5:1 Z:E mixture in 57% yield. Hydrogenation of **37** (H_2/$Pd(OH)_2$) results in a complex mixture of products, from which the 1,2 diamino ester **38** is anyway isolated in 40% yield. The formation of **38** may result from initial N-debenzylation of **37** followed by intramolecular Michael addition resulting aziridine. The problem can be partly circumvented by performing the hydrogenation starting from the acid **39**. Treatment of **39** with H_2/Pd-C followed by reflux in MeOH gives an easily separable 1:1 mixture of **1a** and **7a** in 40% yield.

[0073] An alternative synthesis of these lactams is also provided starting from the trifluoroacetamido aldehyde **13** (Figure 6). Aldehyde **13** is synthesised from **36** with a series of 5 high-yielding steps. Horner-Emmons and nitrogen protection gives **40** (46% over 7 steps), which could be directly reduced to give a 1:1 mixture of the fully protected ester **41** (77%). Removal of the trifluoroacetamido protecting group ($NaBH_4$ in MeOH, 84%) followed by treatment in refluxing xylene gives the lactams **1a** and **7a** in 78% yield.

[0074] The same synthetic schemes are equally adopted for the synthesis of the "trans" lactam series.

[0075] Starting material for the 6,5-fused "trans" lactams **5a** and **11a** is the trans-substituted proline **17** (Figure 7). Aldehyde **17** is best obtained from ester **43**, which is made in one step from N-Cbz-5-hydroxy proline tert-Butyl ester as 4:1 trans:cis mixture, following a published procedure. (I. Collado et al., Tetrahedron Lett., 1994, 43, 8037) The Horner-Emmons reaction with the potassium enolate of **26** proceeds with 98% yield. Treatment with Boc_2O and cis/trans isomers separation, followed by unselective H_2/Pd-C hydrogenation of the crude and treatment in refluxing MeOH gives a 1:1 mixture of easily separated **5a** and **11a**.

[0076] Finally, synthesis of the 7,5-fused "trans" lactams **6a** and **12a** is achieved starting from the "trans" allyl proline

45 (Figure 8). (M. V. Chiesa et al. Synlett 1996, 441-443) Hydroboration and Swern oxidation (80% over 2 steps) gives the aldehyde **18**, which reacted with **26** to give, after nitrogen protection, **46** as a 6:1 Z:E mixture. The usual sequence (NaOH; H_2/Pd-C) allowed the isolation of **6a** and **12a** in 40% overall yield.

[0077] As far as the synthesis of the cyclic RGD portion, synthetic methods are well known in the art. It is convenient to use the solid phase synthesis approach, although other methods could be used.

[0078] The classical solid-phase synthesis is preferred.

[0079] The solid-phase synthesis is carried out as outlined in C.Gennari et al. Eur.J.Org. Chem. **1999**, 379-388.

[0080] The protected amino acid is condensed on a suitable resin, for example a Wang-Merrifield resin. Protecting groups are known in this art. 9-fluorenylmethoxycarbonyl (FMOC) is preferred

[0081] After having activated the resin, N-FMOC-Gly is attached to the Wang-Merrifield resin by means of a suitable condensing agent, preferably diisopropylcarbodiimide (DIC)/1-hydroxybenzotiazole (HOBt)/4-dimethylaminopyridine (DMAP) (J.Org. Chem, **1996**, 61, 6735-6738.

[0082] Subsequently, N-FMOC-Arg(Pmc)OH is attached, followed by the bicyclic N-FMOC-lactam (IIIa) or (IIIb) and finally N-FMOC-Asp(tBu)OH.

[0083] In a still preferred embodiment of the present invention, the solid phase synthesis of cyclic peptides containing the RGD sequence bonded to the bicyclic lactam was performed with 9-flourenylmethoxy carbonyl (Fmoc) strategy. Thus the N-Boc protecting group had to be exchanged by Fmoc group in the bicyclic lactam. The synthesis was performed using Merrifield solid phase peptide synthesis with SASRIN (Super Acid Sensitive Resin) applying Fmoc strategy. Asp was protected at the carboxy group in the side chain as t-butylester and Arg was protected at the guanidino group as Pmc (2,2,5,7,8 Pentamethyl chroman-6 sulphonyl). Linear polipeptides were assembled leaving the glycine residue at the C-terminus to prevent racemization and steric hindrance during the cyclization step. The Fmoc group was cleaved with 20% piperidine in DMF. The Fmoc-protected aminoacid and bicyclic lactams were coupled with HOAT (Azahydroxy Benzotriazole) in the presence of DIC (Diisopropylcarbodiimide) or with HOAT/HATU {Azahydroxy Benzotriazole)/{O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronioesafluorophosphate} using collidine as base. Peptides were cleaved from SASRIN solid support by 1% TFA in DCM and subsequent neutralisation of TFA with Py. This procedure leads to peptides with intact side chain protective groups. Final cyclization was performed in the same conditions i.e. HOAT/HATU and final deprotection was done with trifluoroacetic acid in the presence of scavengers to avoid side alkylations.

[0084] The compounds of the present invention are endowed with interesting physiological properties, which make them useful as medicaments. In particular, the compounds of formula (I) herein disclosed are selective antagonists of $\alpha_v\beta_3$ integrins. This antagonist activity provides the use of said compounds for the preparation of medicaments useful in inhibiting the action of $\alpha_v\beta_3$ integrins. In particular, said medicaments will be used in the treatment of tumors, namely in inhibiting tumor growth and/or angiogenesis or metastasis.

Receptor Binding Assay

[0085] By way of example, the tests were performed on the preferred compound ST 1646 (see claim 9) and for comparison purposes, the highly active compound of the prior art, namely c(RGDfV), i.e. cyclo (Arg-Gly-Asp-D-Phe-Val), in the attached report named as the "KESSLER" peptide, disclosed in WO 9706791was used. Both ST 1646 and "KESSLER" are also named "RGD".

Materials And Methods.

[0086] The receptor binding assay was performed as described by Orlando and Cheresh *(Arginine-Glycine-Aspartic Acid Binding Leading to Molecular Stabilization between Integrin $\alpha_v\beta_3$ and Its Ligand. J.* Biol. Chem. **266**: 19543-19550, 1991). $\alpha_v\beta_3$ was diluted at 500 ng/ml in coating buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$) and an aliquot of 100 µl/well was added to a 96-well microtiter plate and incubated overnight at 4°C. The plate was washed once with blocking/binding buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1% bovine serum albumin), and incubated an additional 2 h at room temperature. The plate was rinsed twice with the same buffer and incubated with radiolabelled ligand at the indicated concentrations. For competition binding, unlabelled competitor and competing peptides were included at the concentration described. After additional three washing, counts were solubilized with boiling 2N NaOH and subjected to γ-counting.

Cell Culture

[0087] Bovine microvascular endothelial cells (BMEC) were maintained in DMEM supplemented with 20% foetal calf serum, 50 units/ml heparin, 50 µg/ml bovine brain extract, 100 units/ml gentamycin.

[0088] BMEC were cultured on 1% gelatine-coated culture flasks and employed in experiments between passage

6-12.

[0089] Human prostate carcinoma cells (PC3) were purchased from American Type Collection Culture (ATCC) and maintained in RPMI supplemented with 10% foetal calf serum, 10 mM L-glutamine, 1% sodium piruvate and 100 units/ml gentamicin.

[0090] Murine lung carcinoma cells (M109) were purchased from American Type Collection Culture (ATCC) and maintained in RPMI supplemented with 10% foetal calf serum, 10 mM L-glutamine and 100 units/ml gentamicin.

[0091] Cells were passaged and used for the experiments before reaching confluence.

Adhesion test

[0092] Ninety-six-well plates (Falcon) were coated with either fibronectin or vitronectin (both at 5 g/ml in phosphate buffered saline) overnight at 4°C. Cells were detached using EDTA (1mM)/ trypsine (0,25%) and resuspended in own medium described above. Approximately 40.000 cells/100 1 were applied for each well and allowed to adhere for 60 min at 37°C in presence of different amounts of RGD peptides. For all experiments the non-adherent cells were removed with PBS and the remaining cells were fixed with 4% paraformaldehyde for 10 min.

[0093] Cells were stained with 1% toluidine blue for 10 min and rinsed with water.

[0094] Stained cells were solubilized with 1% SDS and quantified on a microtiter plate reader at 600nm.

[0095] Experiments described were performed in quadruplicate and repeated a minimum of three times.

[0096] Results were presented as mean and standard deviation.

Results

Binding Assay

[0097] Both purified and membrane-bound integrin $\alpha_v\beta_3$ bind to the disintegrin echistatin with high affinity, which can be competed efficiently by linear and cyclic RGD peptides (C.C. Kumar, Huimingnie, C.P. Rogers, M. Malkowski, E. Maxwell, J.J. Catino and L. Armstrong. 1997, The Journal of Pharmacology and Experimental Therapeutics; (283) pp 843-853). Therefore to assess the affinity of these peptides for this integrin we used an experimental protocol of competition with the [^{125}]I-echistatin as described in materials and methods.

[0098] Our results are showing that ST1646 (the compound of claim 9) is the more effective peptide to shift echistatin from its interaction with the $\alpha_v\beta_3$ integrin. Indeed affinity of the RGD peptide ST1646 reported in table 2 as IC_{50} of the binding concentration was almost 20 time higher than the Kessler cyclic peptides used as reference peptide. Therefore these data are providing clear evidence that the structural constrain of the RGD sequence introduced by the ST 1646 result unexpectedly in an affinity for the $\alpha_v\beta_3$ integrin notably higher than Kessler peptide.

Table 2

Competition binding of RGD to Integrin $\alpha_v\beta_3$ Receptor		
RGD	IC_{50} ±SD (nM)	Ki ±SD (nM)
KESSLER RGD	36.9 ±6.4	34.06 ±5.9
ST 1646	2.2 ±0.32	2.03 ±0.29

[0099] Effect of RGD compounds on the binding of [125I] Echistatin to $\alpha_v\beta_3$ integrin.

[0100] IC_{50}, the concentration of compounds required for 50% inhibition of echistatin binding, were estimated graphically by program Allfit. The Ki of the competing ligands were calculated according to the Cheng and Prusoff equation.

[0101] Values are the mean ±standard deviation of triplicate determinations.

[0102] Saturation binding isotherms of ^{125}I-echistatin binding to $\alpha_v\beta_3$ receptor were determined in a solid-phase receptor binding assay as described in materials and method. Integrin $\alpha_v\beta_3$ was coated and incubated with various concentrations (0.05-10 nM) of ^{125}I-echistatin. Non specific binding was evaluated by carrying out the binding assay in the presence of an excess of cold echistatin and was subtracted from the total binding to calculate specific binding.

[0103] In competition binding ^{125}I-echistatin was added to the wells to a final concentration of 0.05 nM in binding buffer in the presence of competing ligand. Cold unlabelled echistatin and peptides dissolved in binding buffer at concentrations ranging between 10^{-4} M to 10^{-9}.

Endothelial Cells Adhesion Assay

[0104] Since transmembrane αβ integrins family are involved in adhesion of endothelial cells to extracellular matrix

proteins we assayed adhesion inhibition of bovine microvascular endothelial cells (BMEC) to both vitronectin and fibronectin when these cells were treated with different concentration of our cyclic RGD.

[0105] According to the binding experiment the cyclic RGD peptide ST1646 was the more effective in inhibiting adhesion than the other peptide tested. Since vitronectin is a more specific ligand of $\alpha_v\beta_3$ integrin than fibronectin we observed that the RGD tested were able to more efficiently inhibit adhesion of BMEC cells on vitronectin than on fibronectin coated plates (Compare Table 3 with Table 4). Comparing adhesion inhibition, we observed that the cyclic RGD ST1646 was about 10 time more effective than the Kessler peptide inhibiting adhesion of BMEC cells to both fibronectin and vitronectin (see Table 5).

[0106] To asses the ability of ST1646 peptide to compete with vitronectin in adhesion assay also on other cells type, we performed this experiment using microvascular endothelial cells (HMEC), human prostate carcinoma cells (PC3) and murine lung carcinoma cells (M109). Table 6 (a, b and c) show a good activity of the ST1646 peptide in inhibiting adhesion of all cells type. Indeed the reported adhesion inhibition of the ST1646 on HMEC, PC3 and M109 cells have shown higher percentage than the Kessler RGD peptide.

[0107] Putting together these data we have, therefore, showed high activity of the RGD cyclic peptide ST 1646 on several cellular type coherently with binding affinity experiment previously described.

Table 3

Adhesion inhibition of BMEC to Vitronectin		
RGD	% inhibition	t-test versus control
KESSLER	96	P<0.0001
ST 1646	99	P<0.0001

[0108] The percentages of adhesion inhibition refer to 100 M concentration of each peptide of and it's calculated by the following formula (control -sample/ control x 100) where control was RGD untreated sample. Each percentage is the mean of 4 independent samples treated with the same peptide. The t-test has been calculated, using the Mann Witney non parametric test, by the instat program

Table 4

Adhesion inhibition of BMEC to Fibronectin		
RGD	% inhibition	t-test versus control
KESSLER	30	P<0.0001
ST 1646	60	P<0.0001

[0109] The percentages of adhesion inhibition refer to 100 μM concentration of each peptide of and it's calculated by the following formula (control-sample/ control x 100) where control was RGD untreated sample. Each percentage is the mean of 4 independent samples treated with the same peptide. The t-test has been calculated, using the Mann Witney non parametric test, by the instat program.

Table 5

IC50 of adhesion inhibition of BMEC		
RGD	IC_{50} (μM)	
	Fibronectin	Vitronectin
KESSLER	>100	7.8±1.2
ST 1646	44 ±4	0.8 ±0.06

[0110] Several concentrations (in quadruplicate) of the indicate RGD peptides ranging between 100 to 0.6 μM has been tested in adhesion experiment as described in materials and methods. The IC_{50} which represent the RGD peptide concentration able to inhibit 50% of the adhesion of BMEC to the indicate substrate, has been calculate by the linear regression analysis using the Allfit program. The IC_{50} for each RGD has been reported together with the standard deviation.

Table 6 (a)

Adhesion assay on Vitronectin

	HMEC CELLS		
RGD	% Inhibition	IC 50 (μM)	t-test versus control
KESSLER	39	4.23±0.31	P<0.01
ST 1646	58	1.27±0.375	P <0.0005

[0111] Serial concentrations (in quadruplicate) of indicated RGD peptide over a wide range (0.01-100μM has been tested in adhesion test, on vitronectin, as described in material and methods.

[0112] The ICso represents the average value of 3 experiments and indicates that RGD peptide concentration able to inhibit the 50% of cell adhesion.

[0113] The percentages of adhesion inhibition refer to 1.5 μM concentration of each peptide and were calculated by the following formula (control-sample/ control x 100) where control was RGD untreated sample.

Table 6 (b)

Adhesion assay on Vitronectin

	PC3 CELLS		
RGD	% Inhibition	IC 50 (μM)	t-test versus control
KESSLER	69	2.5±0.2	P<0.0001
ST 1646	96	0.3±0.08	P<0.0001

[0114] Serial concentrations (in quadruplicate) of indicated RGD peptide over a wide range (0.01-100μM has been tested in adhesion test, on vitronectin, as described in material and methods.

[0115] The IC_{50} represents the average value of 3 experiments and indicates that RGD peptide concentration able to inhibit the 50% of cell adhesion.

[0116] The percentages of adhesion inhibition refer to 1.5 μM concentration of each peptide and were calculated by the following formula (control-sample/ control x 100) where control was RGD untreated sample.

Table 6 (c)

Adhesion assay on Vitronectin

	M109 CELLS		
RGD	% Inhibition	IC 50 (μM)	t-test versus control
KESSLER	70	0.46±0.5	P<0.0001
ST 1646	99	0.048±0.06	P<0.0001

[0117] Serial concentrations (in quadruplicate) of indicated RGD peptide over a wide range (0.01-100μM has been tested in adhesion test, on vitronectin, as described in material and methods.

[0118] The IC_{50} represents the average value of 3 experiments and indicates that RGD peptide concentration able to inhibit the 50% of cell adhesion.

[0119] The percentages of adhesion inhibition refer to 1.5 μM concentration of each peptide and were calculated by the following formula (control-sample/ control x 100) where control was RGD untreated sample.

[0120] The t-test has been calculated using the Mann Witney non parametric test, by the instat program. In the top left side of the two panels it's shown the cell type the adhesion experiment it's referred to.

Antitumor And Antimetastatic Activity Of St 1646 Vs. Kessler Peptide On M109 Lung Carcinoma-Bearing Balb/ C Mice

[0121] Balb/c mice were injected i.m. with M109 lung carcinoma cells ($3x10^5$ cells/mouse) into the hind leg muscle. One day after tumor injection, mice were treated with ST 1646 (300 μg/mouse = 15 mg/kg) or Kessler peptide (200 μg/mouse = 10 mg/kg) according to a qdx9 treatment schedule (every day for 9 administration, i.p. route).

[0122] Tumors were excised at day 10^{th} after tumor implant. Mice were sacrificed at day 16^{th} from tumor implant and lungs were removed. The number of lung metastases has been evaluated on tumor-excised mice (3 mice/group) using a dissecting microscope.

[0123] TVI % (tumor volume inhibition) = 100 - [(mean tumor weight of treated group/mean tumor weight of control group) x 100]. Calculated on day 16^{th} after tumor implant (just before mice sacrifice) on nonoperated mice.

[0124] The results obtained, reported in table 7, shown that ST1646 is more effective than Kessler peptide in reducing both the number of the metastasis and the volume of the tumor.

Table 7

Antitumor and antimetastatic activity of ST 1646 vs. Kessler peptide on M109 lung carcinoma-bearing Balb/c mice.			
Group	Schedule	Mean no. of metastases	TVI %
Untreated	/	34	/
Kessler 200 μg/mouse (10 mg/kg)	qdx9	23	/
ST 1646 300 μg/mouse (15 mg/kg)	qdx9	20	3

Angiogenesis Inhibition On Cam Assay With St 1646 Cyclopeptide

[0125] Angiogenesis on CAM (chicken embryo chorioallantoic membrane) assay has been quantified by counting the number of vessels interfacing the implanted gelatin sponge on each embryos and calculating the average for each single experimental point (6-8 eggs for peptide concentration). A single treatment means that the embryo received the peptide, at the concentration indicated in the table, only one times at the beginning of the experiment while in the repeated treatment the peptide has been added to the embryo every day for three days. In some experiments we have

refereed our sample to control where angiogenesis occurred spontaneously on the chorioallantoic membrane during embryo development (Table 8). In others experiment (Table 9) instead we have used control where angiogenesis has been stimulated by bFGF (400ng/embryo).

Table 8

Angiogenesis inhibition occurred spontaneously on the chorioallantoic membrane		
Treatment	Inhibition (%)	Standard Deviation (%)
Control	0	
ST1646 (100 μg single treatment)	-70	±27
ST1646 (20 μg repeated treatment)	-27	±8
Inhibition (%) = [(mean vessels treated group - mean vessels control group) / control group] x 100		

Table 9

Angiogenesis inhibition on the chorioallantoic membrane where angiogenesis has been stimulated by bFGF.		
Treatment	Inhibition (%)	Standard Deviation (%)
Control bFGF (400ng)	0	
ST1646 (100μg single treatment)	-56	±18
ST1646 (100μg repeated treatment)	-84	±30
Inhibition (%) = [(mean vessels treated group - mean vessels control group)/control group] x 100		

[0126] The results obtained provide a clear evidence that the structural constrain of the RGD sequence introduced by the ST 1646 result unexpectedly in an affinity for the $\alpha_v\beta_3$ integrin notably higher than Kessler peptide. Paralleled to these results in *in vitro* competition binding assay, ST 1646 assesses its activity in inhibiting the binding of several cell types to fibronectin and vitronectin proteins [table 3-4-5-6(a. b and c)]. According to the binding assay experiments (Table 3), cellular inhibition assay show that ST 1646 is at least 10 folds more active than Kessler peptide. Moreover, ST ST 1646 is extremely specific in inhibiting cellular binding to vitronectin. This is an additional evidence, in which ST 1646 shows a good selectivity towards cellular $\alpha_v\beta_3$ integrin implicated in binding to vitronectin substrates. In *in vivo* experiments the results obtained shown that ST 1646 inhibits the growth of M109 lung metastasis (table 7). In addition, ST 1646 strongly inhibits angiogenesis both in FGF-induced and spontaneous angionenesis (table 8 and 9 respectively). This results show that ST 1646 is a very effective antitumoral and antiangiogenic compound.

[0127] The compounds of the present invention have azabicycloalkane structure and contain the RGD (Arg-Gly-Asp) sequence are selective inhibitors of αvβ3 receptor, and they are useful agents for treating pathologies due to an altered activation of the αvβ3 receptor. It is well known that the activation of αvβ3 receptor is linked to several pathological processes.

[0128] As above mentioned, the experimental results above reported shown that compounds according to the invention are/have: selective inhibitor of αvβ3 receptor; inhibitors of the adhesion of cell lines to fibronectin; antitumoral activity (reduction of the number of the metastasis); antiangiogenetic activity.

[0129] As far as the industrial aspects of the present invention are concerned, the compounds of formula (I) shall be suitably formulated in pharmaceutical compositions. Said compositions will comprise at least one compound of formula (I) in admixture with pharmaceutically acceptable vehicles and/or excipients. According to the therapeutic necessity, the bioavailability of the selected compound, its physico-chemical characteristics, the pharmaceutical compositions according to the present invention will be administered by enteral or parenteral route. Enteral pharmaceutical compositions may be both in the liquid or solid from, for example tablets, capsules, pills, powders, sachets, freeze dried powders to be readily dissolved or in any other way soluble powders, solutions, suspensions, emulsions. Parenteral formulation will be in injectable form, as solutions, suspensions, emulsions or in powdery form to be dissolved immediately before use. Other administration routes are also provided for example intranasal, transdermal or subcutaneous implant. Special pharmaceutical compositions can also be provided. For example controlled release formulations or particular vehicles, for example liposomes.

[0130] The preparation of the pharmaceutical compositions according to the present invention is absolutely within the general knowledge of the person skilled in this art.

[0131] The dosage will be established according to the type of the pathology to be treated, its severity, and the

conditions of the patient (weight, age, and sex).

[0132] The following examples further illustrate the invention.

[0133] Examples 1-12 may be read easier by making reference to Figures 1-8

[0134] General: ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or C_6D_6 as indicated, at 200 (or 300) and 50.3 MHz, respectively. The chemical shift values are given in ppm and the coupling constants in Hz. Optical rotation data were obtained on Perkin-Elmer model 241 polarimeter. Thin-layer chromatography (TLC) is carried out using Merck precoated silica gel F-254 plates. Flash chromatography is carried out with Merck Silica Gel 60, 200-400 mesh. Solvents were dried with standard procedure, and reactions requiring anhydrous conditions were performed under a nitrogen atmosphere. Final product solutions were dried over Na_2SO_4, filtered and evaporated under reduced pressure on a Buchi rotary evaporator.

Example 1

Preparation of enamides via Horner-Emmons reaction.

General procedure A:

[0135] To a stirred solution of tBuOK (7.36 mmol) in 40 ml of dry CH_2Cl_2 under nitrogen atmosphere, at -78 °C, was added a solution of Z-α-phosphonoglycine trimethyl ester **26** (7.36 mmol) in 5.0 ml of dry CH_2Cl_2. The solution was stirred for 30 min at this temperature and then a solution of aldehyde (6.13 mmol) in dry CH_2Cl_2 (25 ml) was added. After 5 hours the solution was neutralised with a phosphate buffer. The aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The crude was purified by flash chromatography (hexane/ethyl acetate), affording the enamide in a Z:E diastereoisomeric mixture.

Preparation of N-Boc-protected enamide.

General procedure B:

[0136] A solution of enamide (11.0 mmol), $(Boc)_2O$ (22.0 mmol) and a catalytic quantity of DMAP in 40 ml of dry THF, was stirred for 30 min. under nitrogen. The solution was then quenched with 40 ml of water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The crude was purified by flash chromatography (hexane/ethyl acetate), yielding the Boc-protected enamide.

Preparation of alcohol via hydroboration.

General procedure C:

[0137] To a solution of allyl proline (2.34 mmol) in dry THF (4.2 ml) was added a 0.5 M solution of 9-BBN in THF (1.26 mmol). The reaction was stirred for 12 h. and then cooled at 0 °C and, water (0.6 ml), a 3 N solution of NaOH (0.5 ml) and H_2O_2 30% (0.44 ml) were added. The reaction was stirred for 1 h. at room temperature and then refluxed for other 2 h. The aqueous phase was extracted with AcOEt, the collected organic phases were dried over Na_2SO_4, filtered and evaporated under reduced pressure, the crude was purified by flash chromatography (hexane/ethyl acetate), yielding the alcohol as yellow oil.

Preparation of aldehyde via Swern oxidation.

General procedure D:

[0138] To a stirred solution of oxalyl chloride (16.9 mmol) in 35 ml of CH_2Cl_2, cooled at -60° C, were added DMSO (23.1 mmol), alcohol (5.66 mmol) dissolved in 21 ml of CH_2Cl_2, TEA (28.2 mmol). The reaction was warmed at room temperature. After one hour the reaction was washed with 50 ml of water and the aqueous phase was extracted with CH_2Cl_2. The collected organic layers were dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude purified by flash chromatography (hexane/ethyl acetate), yielding the aldehyde.

Example 2

Aldehyde (**14**):

[0139] A stirred solution of **25** (6.0 g, 17.4 mmol) in 84 ml of CH_2Cl_2 was cooled at -60° C and bubbled with O_3 (flow rate = 30 l/hour). After 1.5 hours the reaction was allowed to warm to room temperature and bubbled with N_2 in order to eliminate the excess of O_3. The solution was then cooled at 0 °C with an ice bath and Me_2S (101.8 mmol, 38 ml) was added. After 5 days of stirring at room temperature the solvent was evaporated under reduced pressure and the crude was purified by flash chromatography (hexane/ethyl acetate, 8:2), yielding 4.53 g of **14** (75%) as yellow oil. - $[\alpha]_D^{22}$ =-22.03 (c = 1.27, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ = 1.4-1.5 [2 s, 9 H, $C(CH_3)_3$], 1.6-2.4 (m, 4 H, CH_2-CH_2), 2.4-3.2 (2 m, 2 *H*, CH_2CHO), 4.3-4.5 (m, 2 H, CH_2-C*H*-N, N-CH-COOtBu), 5.15 (s, 2 H, CH_2Ph), 7.30 (m, 5 H, aromatic), 9.8 (2 s, 1 H, *CH*O). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 200.8, 171.7, 154.0, 136.2, 128.3, 128.0, 127.8, 127.6, 81.4, 67.0, 66.9, 60.8, 60.3, 54.0, 53.2, 49.0, 48.3, 31.0, 30.2, 29.5, 28.9, 28.0, 27.7. - FAB$^+$MS: calcd. for $C_{19}H_{25}NO_5$ 347.4, found 348.

Example 3

Enamide (**20**):

[0140] The general procedure A was followed using **14** and the crude was purified by flash chromatography (hexane/ethyl acetate, 65:35), affording **20** (98%) in a 7:1 Z:E ratio as colourless oils. Z-isomer :-$[\alpha]_D^{22}$ = +38.78 (c = 1.26, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.3-1.5 [2 s, 9 H, $C(CH_3)_3$], 1.5-2.3 (m, 4 H, CH_2-CH_2), 2.4-2.7 (2 m, 2 H, =CH-CH_2), 3.7 (2 s, 3 H, $COOCH_3$), 4,2 (2 m, 2 H, -CH_2-C*H*-N, N-C*H*-COOtBu), 5.10 (m, 4 H, CH_2Ph), 6.15 (m, 1 H, =*CH*), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 172.4, 164.9, 154.5, 136.2, 132.5, 128.3, 128.2, 127.8, 127.7, 127.6, 81.8, 67.2, 66.9, 60.8, 60.3, 57.9, 57.2, 52.1, 33.8, 33.2, 30.7, 29.8, 29.5, 29.0, 28.0, 27.7, 27.6. - FAB$^+$MS: calcd. for $C_{30}H_{36}N_2O_8$ 552.6, found 553. - E-isomer :- $[\alpha]_D^{22}$ =-4.08 (c = 1.17, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.25-1.50 [3 s, 9 H, $C(CH_3)_3$], 1.5-2.3 (m, 4 H, CH_2-CH_2), 2.8-3.3 (2 m, 2 H, =CH-CH_2), 3.8 (2 s, 3 H, $COOCH_3$), 4,1 (m, 1 H, -CH_2-C*H*-N), 4.25 (m, 1 H, N-CH-COOtBu), 5.15 (2 s, 4 H, CH_2Ph), 6.30 (m, 1 H, =CH), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.8, 164.4, 154.1, 153.6, 136.4, 135.9, 128.7, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 126.5, 125.9, 81.2, 80.9, 66.7, 61.0, 60.6, 60.2, 58.8, 58.1, 52.2, 32.7, 32.0, 31.8, 29.9, 29.5, 29.2, 28.8, 27.8, 27.7, 22.5, 14.0.

Example 4

Enamide (**27**):

[0141] The general procedure B was followed using **20** and the resulting crude was purified by flash chromatography (hexane/ethyl acetate, 7:3), yielding 27 (98%) as yellow oil. - Z-isomer: - $[\alpha]_D^{22}$ = +16.95 (c = 1.86, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.3-1.5 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.2 (m, 4 H, CH_2-CH_2), 2.3-2.8 (2 m, 2 H, =CH-CH_2), 3.7 (s, 3 H, $COOCH_3$), 4,1-4.2 (2 m, 2 H, =CH-CH_2-C*H*-N, N-CH-COOtBu), 5.15 (m, 4 H, CH_2Ph), 6.95 (dd, J = 8.5, J = 6.4 Hz, 1 H, =*CH*), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.4, 163.8, 154.6, 154.3, 152.1, 150.4, 139.0, 138.8, 136.2, 135.1, 129.7, 128.3, 128.2, 128.1, 127.8, 127.6, 83.3, 81.2, 77.1, 68.2, 66.8, 60.9, 60.4, 57.5, 56.7, 52.1, 32.8, 32.1, 29.9, 29.1, 28.8, 27.7. - E-isomer: - $[\alpha]_D^{22}$ = +7.34 (c = 1.33, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.3-1.5 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.2 (m, 4 H, CH_2-CH_2), 3.0-3.3 (m, 2 H, =CH-CH_2), 3.75 (2 s, 3 H, $COOCH_3$), 4,1-4.2 (2 m, 2 H, =CH-CH_2-C*H*-N, N-CH-COOtBu, 5.1-5.2 (m, 4 H, CH_2Ph), 6.3 (m, 1 H, =*CH*), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.6, 163.8, 154.5, 154.3, 152.1, 150.4, 142.8, 142.5, 136.3, 135.2, 128.7, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 83.2, 81.1, 68.2, 66.8, 61.1, 60.6, 58.1, 57.4, 51.7, 32.7, 32.0, 29.5, 29.4, 28.9, 28.7, 27.7..

Example 5

6,5-Fused bicyclic lactam (**2a, 8a**):

[0142] A solution of 0.320 g of **27** (0.49 mmol) and a catalytic quantity of Pd/C 10% in 5 ml of MeOH was stirred under H_2 for one night. The catalyst was then filtered through celite and the filtration bed was washed with MeOH. The solvent was evaporated under reduced pressure, the residue was dissolved in MeOH and refluxed for 48 h. The solvent

was removed and the two diastereoisomers formed were separated by flash chromatography (hexane/ethyl acetate, 7:3), yielding 0.122 g of **8a** and **2a** (70%) in a 1.4:1 diastereoisomeric ratio as white foam. - $[\alpha]_D^{22}$ = -10.70 (c = 1.29, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$): δ = 1.43-1.45 [2 s, 18 H, $C(CH_3)_3$], 1.5-2.5 (m, 8 H, CH_2-CH_2, BocN-CH-CH_2-CH_2), 3.69 [m, 1 H, CH-N], 4.1 (m, 1 H, CH-NBoc), 4.38 (dd, J = 7.7 Hz, J = 1.8 Hz, 1 H, N-CH-COOtBu), 5.59 (d, J = 5.4 Hz, 1 H, N*H*). - ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 170.7, 165.8, 155.8, 147.1, 81.4, 79.3, 59.0, 56.2, 49.9, 32.0, 29.5, 29.1, 28.2, 27.8, 27.0, 26.5. - FAB+MS: calcd. for $C_{18}H_{32}N_2O_5$ 354.46, found 354. - 8a - $[\alpha]_D^{22}$ = -45.07 (c = 1.69, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$): δ = 1.44-1.46 [2 s, 18 H, $C(CH_3)_3$], 1.55-2.2 (m, 7H, CH_2-CH_2, BocN-CH-CHH-CH_2), 2.5 (m, 1H, BocN-CH-CH*H*), 3.75 [tt, J = 11.2 Hz, J = 4.2 Hz, 1 H, CH-N], 3.90 (m, 1 H, CH-NBoc), 4.32 (d, J = 9.2 Hz, 1 H, N-CH-COOtBu), 5.59 (broad, 1 H, N*H*). - ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 170.6, 167.9, 155.7, 81.2, 79.4, 77.5, 60.4, 59.0, 52.2, 31.4, 28.5, 28.3, 28.2, 27.8, 27.6. - FAB+MS: calcd. for $C_{18}H_{32}N_2O_5$ 354.46, found 354.

Acid **(28)**:

[0143] To a solution of **27** (0.640 g, 0.980 mmol) in 4.9 ml of MeOH was added 4.9 ml of 1N NaOH (4.9 mmol). After 18 hours of stirring at room temperature the solvent was evaporated under reduced pressure. The solid residue was dissolved in 5 ml of water and 2N HCl was added until pH 3, then the aqueous solution was extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4, the solvent evaporated under reduced pressure and the crude was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5), yielding 0.420 g of 28 (85%) as a white solid.

[0144] Z isomer: - $[\alpha]_D^{22}$ = -57.01 (c = 1.99, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.30-1.50 [2 s, 18 H, $C(CH_3)_3$], 1.7-2.7 (m, 6 H, CH_2-CH_2, =CH-CH_2), 4.2-4.3 (m, 2 H, =CH-CH_2-C*H*-N, N-CH-COOtBu), 5.1 (m, 2 H, CH_2Ph), 6.6 (m, 1 H, =*CH*), 7.30 (m, 6 H, aromatic, NHBoc). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.5, 168.3, 154.8, 154.5, 140.6, 136.4, 136.1, 133.9, 133.5, 128.3, 128.2, 128.1, 127.8, 127.4, 126.9, 81.3, 80.9, 67.1, 66.9, 65.0, 66.9, 65.0, 57.5, 56.8, 33.4, 32.4, 29.5, 28.5, 28.5, 28.0, 27.8, 27.7, 27.4.

[0145] E isomer: - $[\alpha]_D^{22}$ = -41.63 (c = 1.87, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.35-1.50 [3 s, 18 H, $C(CH_3)_3$], 1.7-2.4 (m, 4 H, CH_2-CH_2), 2.7-3.2 (m, 2 H, =CH-CH_2), 4,2-4.3 (m, 2 H, =CH-CH_2-C*H*-N, N-CH-COOtBu), 5.1 (m, 2 H, CH_2Ph), 6.7-6.9 (m, 2 H, =CH, NHBoc), 7.30 (m, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.7, 167.2, 154.9, 154.5, 154.3, 136.5, 136.2, 128.3, 128.2, 127.7, 127.5, 126.9, 126.3, 126.1, 81.2, 80.4, 66.9, 65.0, 60.7, 60.4, 58.3, 57.7, 32.9, 32.0, 29.5, 28.4, 28.1, 27.8, 27.7, 27.4, 27.1, 14.0.

Acid (32, 33):

[0146] To the [Rh-(-)-BitianP] catalyst prepared as described in the literature was added **28** (0.16 mmol) and MeOH (30 ml), the resulting solution was stirred for 30 min. A 200 ml stainless-steel autoclave equipped with a magnetic stirrer and a thermostatic bath was pressurised with hydrogen and vented three times. The solution was transferred into the autoclave with a syringe and the autoclave was pressurised at 10 KPa with hydrogen. The solution was stirred for 24 h. at 30 °C. The hydrogen pressure was released, the solvent evaporated. The crude was submitted to the next reaction without further purification.

6,5-fused bicyclic lactam **(2a)**:

[0147] To a solution of **32** and **33** as diastereomeric mixture in MeOH (1.5 ml) was added a solution of CH_2N_2 in Et_2O until the TLC showed that the reaction was complete. The solution was evaporated and the crude was dissolved in MeOH (2 ml) and a catalytic quantity of Pd/C was added, the mixture was stirred under H_2 for 12 h. The catalyst was then filtered through celite pad and washed with MeOH. The solvent was evaporated under reduced pressure and the crude, as a white foam, was refluxed in MeOH for 48 h. The solvent was evaporated under reduced pressure and the crude was purified by flash chromatography (hexane/ethyl acetate 7:3) affording **2a** (85%) as a white solid.

Example 6

6,5-fused bicyclic lactam **(8a)**:

[0148] This bicyclic lactam was achieved with the same synthetic sequence followed for the lactam **2a** using for the asymmetric hydrogenation the [Rh-(+)-BitianP] catalyst.

Aldehyde (15):

[0149] The general procedure C was followed using **25** and the resulting residue was purified by flash chromatography (hexane/ethyl acetate, 7:3), yielding the alcohol (95%) as yellow oil.-^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.4 [s, 9 H, $C(CH_3)_3$], 1.6-2.4 (m, 8 H, CH_2-CH_2), 3.5-3.8 (2 m, 2 H, CH_2OH), 4.1 (m, 1 H, CH_2-CH-N), 4.25 (m, 1 H, N-CH-COOtBu), 5.15 (s, 2 H, CH_2Ph), 7.30 (m, 5 H, aromatic).

[0150] The general procedure D was followed using the previous alcohol and the resulting crude residue was purified by flash chromatography (hexane/ethyl acetate, 7:3), yielding 15 (89%) as an oil. - ^{1}H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ = 1.4-1.5 [2 s, 9 H, $C(CH_3)_3$], 1.6-2.8 (m, 4 H, CH_2-CH_2), 4.05 (m, 1 H, CH_2-CH-N), 4.25 (m, 1 H, N-CH-COOtBu), 5.15 (s, 2 H, CH_2Ph), 7.30 (m, 5 H, aromatic), 9.6-9.8 (2 s, 1 H, CHO).

Aminoester (34):

[0151] The general procedure A was followed using **15** and the resulting residue was purified by flash chromatography yielding the enamide (95%) as yellow oil. The compound previously synthesised was submitted to the general procedure B and the resulting residue was purified by flash chromatography yielding the N-Boc protected compound (95%) as white solid. A solution of this compound (0.96 mmol) in MeOH (1 mL) and a catalytic quantity of Pd/C were stirred under hydrogen atmosphere for 12h. The catalyst was then filtered through a celite pad. The solvent was evaporated under reduced pressure yielding 0.320 g of **34** (83%) as a white solid (mixture of two diastereoisomers). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.47, 1.48 [2 s, 18 H, $C(CH_3)_3$], 1.40-2.1 (m, 10 H, CH_2-CH_2, BocN-CH-CHH-CH_2), 3.00 (m, 1 H, CH-N), 3.6 (m, 1 H, N-CH-COOtBu), 4.3 (m, 1 H, CH-NBoc), 5.05 (db, 1H, NH).

Amino acid (**35**):

[0152] To a solution of 34 (0.288 g, 0.720 mmol) in MeOH was added 1N NaOH, after 1.5 h. the solution was acidified until pH 3 with 1N HCl, then the solution was evaporated. The crude was submitted to the next reaction without further purification.

Example 7

7,5-fused bicyclic lactams (**3a, 9a**):

[0153] To a solution of the crude **35** (0.720 mmol) in CH_2Cl_2 (80 ml) was added in the order: Et_3N (0.720 mmol, 0.220 ml), HOBt (0.166 g, 1.22 mmol) and a catalytic quantity of DMAP. After 15 min was added EDC (0.180 g, 0.937 mmol) and the solution was stirred for 24 h. To the solution was added H_2O (40 ml), the aqueous phase was extracted with CH_2Cl_2 and the collected organic layers were dried with Na_2SO_4 filtered and evaporated under reduced pressure affording 0.191 g of **3a** and **9a** in a 1:1 diastereoisomeric ratio and 72% of yield over 2 steps.

[0154] (3a). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.41, 1.42 [2 s, 18 H, $C(CH_3)_3$], 1.5-2.5 (m, 10 H, CH_2-CH_2), 3.80 (m, 1 H, CH-N), 4.2 (m, 1 H, CH-NBoc), 4.51 (dd, J = 4.8 Hz, 1 H, N-CH-COOtBu), 5.54 (db, 1 H, NH). - (9a). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42, 1.43 [2 s, 18 H, $C(CH_3)_3$], 1.50-2.2 (m, 10H, CH_2-CH_2), 3.8 [m, 1 H, CH-N], 4.25 (dd, J = 4.6 Hz, J = 9.6 Hz, 1 H, CH-NBoc), 4.42 (dd, J = 2.3 Hz, J = 7.2 Hz, 1 H, N-CH-COOtBu), 5.30 (bs, 1 H, NH).

[0155] Enamide (**37**): The general procedure D was followed using **36** and the crude was purified by flash chromatography (hexane/ethyl acetate, 7:3), yielding the aldehyde (81%) as an oil. - ^{1}H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ = 1.48 [s, 9 H, $C(CH_3)_3$], 1.8-2.2 (m, 4 H, CH_2-CH_2), 3.21 (m, 1 H, CH_2-CH-N), 3.45 (m, 1 H, N-CH-COOtBu), 3.70 (d, J = 12 Hz, 1 H, HCHPh), 4.10 (d, J = 12 Hz, 1 H, HCHPh), 7.30 (m, 5 H, aromatic), 9.12 (d, 1 H, CHO).

[0156] The general procedure A was followed using the previous aldehyde and the crude was purified by flash chromatography (hexane/ethyl acetate, 65:35), affording the enamide (98%) in a 9:1 Z:E ratio as colourless oils. Z-isomer - ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.31 [s, 9 H, $C(CH_3)_3$], 1.7-2.2 (m, 4 H, CH_2-CH_2), 3.3 (m, 1 H, N-CH-COOtBu) 3.5 (s, 1 H, CH_2-CH-N), 3.66 (d, J = 13.2 Hz, HCHPh) 3.73 (s, 1 H, $COOCH_3$), 3.79 (d 1 H, HCHPh), 5.11 (d, J = 12.5 Hz, 1 H, OHCHPh), 5.15 (d, J = 12.5 Hz, 1 H, OHCHPh), 6.07 (d, J = 7.4 Hz, 1 H, =CH), 7.10-7.6 (m, 10 H, aromatic), 8.15 (sb, 1 H, -NH).-^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 173.7, 165.1, 154.1, 137.4, 136.1, 129.5, 128.5, 128.3, 128.0, 127.8, 127.7, 127.1, 80.5, 66.9, 65.3, 62.3, 57.5, 52.0, 30.1, 28.9, 27.7.

[0157] The general procedure B was followed using the enamide previous synthesised. The crude was purified by flash chromatography (hexane/ethyl acetate, 7:3) yielding **37** (98%) as a white solid. - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.3-1.5 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.2 (m, 4 H, CH_2-CH_2), 3.1 (m, 1 H, N-CH-COOtBu), 3.5 (m, 1 H, CH_2-CH-N), 3.7 (s, 1 H, $COOCH_3$), 3.7 (d, J = 12 Hz, 1 H, HCHPh), 3.9 (d, J = 12 Hz, 1 H, HCHPh), 5.20 (d, J = 12 Hz, 1 H, HCHPh), 7.0 (d, J = 8.6 Hz, 1 H, =CH), 7.1-7.4 (m, 10 H, aromatic).

[0158] Amino acid (**39**): To a solution of **37** (0.424 g, 0.713 mmol) in MeOH (4 ml) was added 1N NaOH (4 mmol, 4 ml) and stirred for 1.5 h. The solution was acidified until pH 3 with 1N HCl, then the solution was evaporated. The crude was submitted to the next reaction without further purification. - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.35, 1.5 [2 s, 18 H, $C(CH_3)_3$], 1.7-2.3 (m, 4 H, CH_2-CH_2), 3.3 (m, 1 H, N-CH-COOtBu), 3.65 (m, 1 H, CH_2-CH-N), 3.7 (d, J = 12.8 Hz, 1 H, HCHPh), 3.9 (d, J = 12.8 Hz, 1 H, HCHPh), 6.5 (d, J = 7.6 Hz, 1 H, =CH), 7.1-7.4 (m, 10 H, aromatic), 9.00 (bs, 1 H, -COOH).

Example 8

5,5-fused bicyclic lactams (**1a, 7a**):

[0159] A solution of **39** (0.713 mmol) and a catalytic quantity of $Pd(OH)_2$/C 20% in 1 ml of MeOH (7 ml) was stirred under hydrogen atmosphere for 12h. The catalyst was then filtered through a celite pad and the solvent was evaporated under reduced procedure. The crude was dissolved in MeOH and refluxed for 48 h. The solvent was evaporated under reduced pressure and the crude was purified by flash chromatography (hexane/ethyl acetate 6:4) affording 0,097 g of **1a** and **7a** as a white solid in 40% of yield (over 2 steps) and 1 : 1 diastereomeric ratio. 1a: - $[\alpha]_D^{22}$ = -4.80 (c = 1.20, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.50, 1.51 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.4 (m, 5 H, CH_2-CH_2, BocN-CH-CHH), 2.95 (m, 1 H, BocN-CH-CHH), 3.85 [m, 1 H, (CH-N], 4.15 (d, J = 8.8 Hz, 1 H, N-CH-COOtBu), 4.60 (m 1 H, CH-NBoc), 5.25 (broad, 1 H, NH). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.7, 169.7, 155.6, 81.8, 79.5, 58.8, 56.5, 56.0, 55.8, 39.5, 33.4, 29.5, 28.2, 27.8. - FAB$^+$MS: calcd. for $C_{17}H_{28}N_2O_5$ 340.41, found 341.-2a: $[\alpha]_D^{22}$ = -4.80 (c = 1.20, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.45 [2 s, 18 H, $C(CH_3)_3$], 1.5-2.5 (m, 6 H, CH_2-CH_2, BocN-CH-CH_2), 4.05 (d, J = 8.8 Hz, 1 H, N-CH-COOtBu), 4.12 (m, 1 H, CH-N), 4.25 (m, 1 H, CH-NBoc), 5.05 (broad, 1 H, NH).- ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 170.9, 169.8, 155.2, 82.2, 81.8, 79.9, 77.1, 61.2, 58.8, 57.6, 56.0, 55.8, 34.4, 33.8, 33.4, 29.9, 29.5, 29.2, 28.5, 28.1, 27.7. - FAB$^+$MS: calcd. for $C_{17}H_{28}N_2O_5$ 340.41, found 341.

Aldehyde (**13**):

[0160] To a stirred solution of **36** (1.5 g, 5.14 mmol) in 39 ml of dry CH_2Cl_2 under nitrogen were added in the order: TBDMSC1 (0.931 g, 6.17 mmol), TEA (6.17 mmol, 0.94 ml) and DMAP (0.063 g, 0.51 mmol). After 12 h. the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (hexane/ethyl acetate, 9:1), yielding 1.910 g of compound (94%) as a colourless oil. - $[\alpha]_D^{22}$ = -3.61 (c = 2.52, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = -0.5 (s, 6 H,CH_3Si), 0.85 [s, 9 H, $(CH_3)_3$C-Si], 1.4 [s, 9 H, $C(CH_3)_3$], 1.5-2.1 (m, 4 H, CH_2-CH_2), 2.9 (m, 1 H, SiO-CH_2-CH-N), 3.3-3.4 (m, 3 H, N-CH-COOtBu, SiO-CH_2), 3.9 (s, 2 H, CH_2Ph), 7.3 (m, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$): δ =173.6, 139.3, 129.1, 127.9, 126.7, 19.9, 67.5, 66.8, 65.8, 58.8, 28.4, 28.0, 27.8, 25.8, 18.1, -3.6.

[0161] A solution of the silyl protected alcohol (1.850 g, 4.55 mmol) and $Pd(OH)_2$/C 20% (0.250 g, 0.45 mmol) in 45 ml of MeOH was stirred under hydrogen atmosphere for 4 hours. Then the catalyst was filtered through celite pad and washed with MeOH, the solvent was evaporated under reduced pressure, yielding 1.34 g of hydrogenated compound (94%) as colourless oil. - $[\alpha]_D^{22}$ = -5.80 (c = 1.99, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) : δ = 0.4 (s, 6 H,CH_3Si), 0.92 [s, 9 H, $(CH_3)_3$C-Si], 1.49 [s, 9 H, $C(CH_3)_3$], 1.5-2.1 (m, 4 H, CH_2-CH_2), 2.35 (broad, 1 H, NH), 3.2 (m, 1 H, SiO-CH_2-CH-N), 3.65 (m, 3 H, N-CH-COOtBu, SiO-CH_2).

[0162] To a stirred solution of the previous compound (1.2 g, 3.79 mmol) in 38 ml of CH_2Cl_2 were added pyridine (11.39 mmol, 0.92 ml) and $(CF_3CO)_2O$ (8.35 mmol, 1.16 ml). After 1.5 hours the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (hexane/ethyl acetate, 9:1), yielding 1.4 g of the N-protected pyrrolidine (89%) as colourless oil. - $[\alpha]_D^{22}$ = -8.62 (c = 2.11, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.4 (s, 6 H, CH_3Si), 0.9 [s, 9 H, $(CH_3)_3$C-Si], 1.47 [s, 9 H, $C(CH_3)_3$], 1.7-2.4 (m, 4 H, CH_2-CH_2), 3.5 (m, 1 H, SiO-CHH), 3.75 (dd, J = 10.6 Hz, J = 4.2 Hz, 1 H, SiO-CHH), 4.2 (m, 1 H, SiO-CH_2-CH-N), 4.35 (t, J = 8.5 Hz 1 H, N-CH-COOtBu).

[0163] To a stirred solution of N-protected pyrrolidine (1.2 g, 2.91 mmol) in 29 ml of THF, cooled at -40° C, was added a 1M solution of TBAF in THF (3.20 mmol, 3.2 ml). Then the solution was allowed to warm at room temp. After 2.5 hours was added 30 ml of brine and the resulting mixture was extracted with ethyl acetate. The organic phase was dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude was purified by flash chromatography (hexane/ethyl acetate, 6:4), yielding 0.850 g of O-deprotected compound (98%) as colourless oil. - $[\alpha]_D^{22}$ = -6.40 (c = 1.45, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.5 [s, 9 H, $C(CH_3)_3$], 2.0-2.4 (m, 4 H, CH_2-CH_2), 3.4-3.7 (m, 2 H, HO-CH_2), 4.2-4.6 (m, 3 H, N-CH-COOtBu, HO-CH_2-CH-N).

[0164] The general procedure D was followed using the alcohol and the residue was purified by flash chromatography (hexane/ethyl acetate, 6:4), yielding the aldehyde (93%) as white solid. - $[\alpha]_D^{22}$ = +22.48 (c = 1.53, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.5 [s, 9 H, $C(CH_3)_3$], 1.8-2.5 (m, 4 H, CH_2-CH_2), 4.5-4.7 (m, 2 H, CHO-CH-N, N-CH-COOtBu), 9.7 (s, 1 H, CHO).

Enamide (**40**):

[0165] The general procedure A was followed using **13** and the crude residue was purified by flash chromatography affording the enamide (68%) as colourless oil (diastereoisomeric ratio Z:E = 1:1). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism and were referred to the mixture of two diastereoisomers): δ= 1.5 [s, 9 H, $C(CH_3)_3$], 1.6-2.45 (m, 4 H, CH_2-CH_2), 3.75 (s, 3 H, $COOCH_3$), 4.6 (m, 1 H, N-C*H*-COOtBu), 4.8 (dd, J = 18 Hz, J = 10 Hz, 1 H, =CH-CH-N), 5.12 (s, 2 H, CH_2Ph), 6.3, 6.8 (2d, J = 10 Hz, 1 H, =CH of Z-isomer, E-isomer), 7.35 (m, 5 H, aromatic).
[0166] The general procedure B was followed using the enamide and the crude was purified by flash chromatography affording 40 with a 95% of yield as colourless oil. - ^{1}H NMR (200 MHz, C_6D_6) (signals were splitted for amidic isomerism and were referred to the mixture of two diastereoisomers): δ= 1.3, 1.5 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.35 (m, 4 H, CH_2-CH_2), 3.7 (s, 3 H, $COOCH_3$), 4.6-4.8 (m, 2 H, N-CH-COOtBu, =CH-CH-N), 5.25 (m, 2 H, CH_2Ph), 7.0 (m, 1 H, =C*H*), 7.35 (m, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, C_6D_6) (signals were splitted for amidic isomerism and were referred to the mixture of two diastereoisomers): δ= 169.1, 163.9, 141.2, 136.1, 129.9, 128.4, 128.2, 127.4, 119.4, 113.7, 83.6, 82.5, 82.0, 68.8, 68.5, 68.2, 62.5, 60.9, 60.8, 58.5, 57.6, 56.8, 53.2, 51.9, 51.7, 51.6, 33.7, 31.8, 30.2, 27.7, 27.5, 26.9.

Aminoester (41)

[0167] A Z/E mixture of 40 (0.609 g, 1.01mmol) and $Pd(OH)_2$/C 20% (0.054 g) in 10 ml of MeOH was stirred under hydrogen atmosphere for 18 h. The catalyst was filtered through a celite pad and washed with MeOH. The solvent was evaporated under reduced pressure and the crude purified by flash chromatography (toluene/Et_2O, 85:15), yielding 0.365 g of 40 (77%) as yellow oil. - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism and were referred to the mixture of two diastereoisomers): δ= 1.45 [s, 18 H, $C(CH_3)_3$], 1.6-2.7 (m, 6 H, CH_2-CH_2, BocN-CH-CH_2), 3.75 (2 s, 3 H, $COOCH_3$), 4.25-4.4 (2 m, 2 H, BocN-CH, BocN-CH-CH_2-C*H*), 4.55 (m, 1 H, N-CH-COOtBu), 5.30 (d, J = 8.5 Hz, 1 H, N*H*). -^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism and were referred to the mixture of two diastereoisomers): δ= 172.4, 170.0, 155.8, 128.9, 128.0, 82.7, 82.0, 79.7, 61.4, 60.6, 58.0, 56.5, 52.2, 51.5, 37.7, 36.4, 35.5, 30.2, 29.7, 29.0, 28.4, 28.1, 27.6, 25.5. - FAB$^+$MS: calcd. for $C_{20}H_{31}F_3N_2O_7$ 468.47, found 468.

Amino acid (42)

[0168] A solution of 41 (0.184 g, 0.393 mmol) and $NaBH_4$ (0.0298 g, 0.781 mmol) in 8 ml of MeOH was stirred for 1 hour at room temperature. The solution was concentrated and 10 ml of water was added. The aqueous solution was extracted with ethyl acetate, the collected organic phases were dried on Na_2SO_4 and the solvent evaporated under reduced pressure. The two diastereoisomers formed in the previous reactions were separated at this step by flash chromatography (ethyl acetate/hexane, 6:4), achieving 0.123 g of 42 (R) and 42 (S) (84%) in a 2.6:1 diastereoisomeric ratio as colourless oil. - 42 (R): - ^{1}H NMR (200 MHz, C_6D_6) (signals were splitted for amidic isomerism): δ= 1.30, 1.45 [2 s, 18 H, $C(CH_3)_3$], 1.5-1.9 (m, 6 H, CH_2-CH_2, BocN-CH-CH_2), 2.85 (m, 1 H, BocN-CH-CH_2-C*H*), 3.2-3.4 (m, 4 H, $COOCH_3$, N-C*H*-COOtBu), 4.65 (m, 1 H, BocN-C*H*), 6.6 (broad, 1 H, N*H*Boc). - ^{13}C NMR (50.3 MHz, C_6D_6) (signals were splitted for amidic isomerism): δ= 174.1, 173.2, 155.8, 81.4, 81.3, 79.5, 60.6, 60.4, 56.5, 56.3, 52.5, 52.0, 37.7, 31.9, 30.0, 29.8, 28.2, 28.0, 27.9. - FAB$^+$MS: calcd. for $C_{18}H_{32}N_2O_6$ 372.46, found 373.-42 (S): - ^{1}H NMR (200 MHz, C_6D_6) (signals were splitted for amidic isomerism): δ= 1.30, 1.50 [2 s, 18 H, $C(CH_3)_3$], 1.50-1.80 (m, 6 H, CH_2-CH_2, BocN-CH-CH_2), 2.8 (m, 1 H, BocN-CH-CH_2-C*H*), 3.3 (s, 3 H, $COOCH_3$), 3.4 (dd, J = 9.1 Hz, J = 5.9 Hz, 1 H, N-C*H*-COOtBu), 4.45 (m, 1 H, BocN-C*H*), 5.3 (broad, 1 H, N*H*Boc). - ^{13}C NMR (50.3 MHz, C_6D_6) (signals were splitted for amidic isomerism): δ= 171.7, 171.5, 164.2, 164.0, 154.7, 154.3, 153.5, 136.6, 136.4, 135.8, 128.4, 128.3, 128.2, 128.1, 127.7, 126.2, 125.9, 125.8, 81.0, 87.1, 66.8, 66.6, 60.8, 60.4, 58.2, 57.5, 52.3, 52.2, 32.8, 31.9, 28.5, 28.1, 27.8, 27.7, 27.4, 27.1. - FAB$^+$MS: calcd. for $C_{18}H_{32}N_2O_6$ 372.46, found 373.

Example 9

5,5-Fused bicyclic lactam [**1a**]:

[0169] A stirred solution of **42** (S) (0.028 g, 0.075 mmol) in 1.5 ml of p-xylene was warmed at 130° C for 24 hours. The solvent was then evaporated under reduced pressure and the crude purified by flash chromatography (hexane/ethyl acetate, 7:3), yielding 19 mg of **1a** (74%) as a white foam. - α_D^{22} = -4.80 (c = 1.20, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ= 1.50, 1.51 [2 s, 18 H, $C(CH_3)_3$], 1.6-2.4 (m, 5 H, CH_2-CH_2, BocN-CH-C*H*H), 2.95 (m, 1 H, BocN-CH-CH*H*), 3.85 [m, 1 H, (CH-N], 4.15 (d, J = 8.8 Hz, 1 H, N-CH-COOtBu), 4.60 (m 1 H, C*H*-NBoc), 5.25 (broad, 1 H, N*H*). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): 6= 171.7, 169.7, 155.6, 81.8, 79.5, 58.8, 56.5, 56.0, 55.8, 39.5, 33.4, 29.5, 28.2, 27.8.-FAB$^+$MS: calcd. for $C_{17}H_{28}N_2O_5$ 340.41, found 341.

Example 10

5,5-Fused bicyclic lactam [**7a**]:

[0170] The compound [**7a**] was achieved from compound **42** (R), by using the same procedure described for the synthesis of compound **1**a, with a 65% of yield as white foam. - $[\alpha]_D^{22}$ = -4.80 (c = 1.20, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ= 1.45 [2 s, 18 H, $C(CH_3)_3$], 1.5-2.5 (m, 6 H, CH_2-CH_2, BocN-CH-CH_2), 4.05 (d, J = 8.8 Hz, 1 H, N-CH-COOtBu), 4.12 (m, 1 H, CH-N), 4.25 (m, 1 H, CH-NBoc), 5.05 (broad, 1 H, NH). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ= 170.9, 169.8, 155.2, 82.2, 81.8, 79.9, 77.1, 61.2, 58.8, 57.6, 56.0, 55.8, 34.4, 33.8, 33.4, 29.9, 29.5, 29.2, 28.5, 28.1, 27.7. - FAB$^+$MS: calcd. for $C_{17}H_{28}N_2O_5$ 340.41, found 341.

Ester (**43**)

[0171] To a stirred suspension of KH (0.777 g, 19.4 mmol) in anhydrous DMF (80 ml) the triethyl phosphonoacetate (19.4 mmol, 3.9 ml) was added. The mixture was stirred at room temperature for 1 h and then a solution of hemiaminal (5.2 g, 16.2 mmol) in DMF (80 ml) was added. The reaction was stirred overnight at room temperature, quenched with satured aqueous NH_4Cl solution and extracted with AcOEt. The combined organic extract were dried over Na_2SO_4 and the solvent was evaporated to dryness and purified by flash chromatography yielding 4.8 g of 43 (75%) in a 4:1 trans:cis diastereoisomeric ratio. - ^{1}H NMR (200 MHz, $CDCl_3$) (signals are splitted for amidic isomerism): δ= 1.2-1.35 (m, 3 H, CH_3CH_2O), 1.35, 1.40, 1.45, 1.50 [4 s, 9 H, $C(CH_3)_3$], 1.60-2.60 (m, 5 H, CH_2-CH_2, $CHCO_2Et$), 2.70-3.1 (2 dd, J_1 = 4 Hz, J_2 = 15 Hz, 1 H, $CHCO_2Et$, trans isomer), 3.2-3.5 (2 dd, J_1 = 4 Hz, J_2 = 15 Hz, 1 H, $CHCO_2Et$, cis isomer), 4.13 (dq, J_1 = J_2 = 7 Hz , 2 H, CH_3CH_2O) 4.27 (m, 1 H, $CHCO_2tBu$), 4.45 (m, 1 H, CH_2-CH-N), 5.15-5.35 (m, 2 H, CH_2Ph), 7.3-7.4 (m, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals are splitted for amidic isomerism): δ= 171.4, 171.3, 171.1, 171.0, 154.4, 154.1, 153.8, 136.5, 136.3, 128.3, 128.2, 127.7, 127.6, 81.2, 66.9, 66.8, 60.8, 60.5, 60.3, 60.2, 55.5, 55.2, 54.5, 39.1, 38.0, 30.4, 29.7, 28.9, 28.7, 28.2, 28.0, 27.8, 27.7, 27.1, 14.1. - FAB$^+$MS: calcd. for $C_{21}H_{29}NO_6$ 391.2, found 392.

Aldehyde (**14, 17**):

[0172] To a stirred solution of **43** (1.205 g, 3.08 mmol) in dry diethylether (31 mL) at -10°C, $LiBH_4$ 2M in THF (1.5 mL, 3.08 mmol) was added. After 24 h a saturated solution of $NaHCO_3$ (40 ml) was added and the resulting mixture was extracted with AcOEt. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash chromatography (hexane/ethyl acetate 1:1), yielding 1.01 g of alcohol (94%) as a yellow oil. - Trans-isomer: $[\alpha]_D^{22}$ = -32.3 (c = 1.02, $CHCl_3$).- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.35 [s, 9 H, $C(CH_3)_3$]. 1.5-2.4 (m, 6 H, CH_2-CH_2, CH_2-CH_2-O), 3.5-3.7 (m, 2 H, CH_2OH), 3.82 (bs, 1 H, OH), 4.22 (dd, J = 7.5, J ~ 0, 1 H, $CHCO_2tBu$), 4.38 (m, 1 H, CH_2-CH-N), 5.15 (m, 2 H, CH_2Ph), 7.32 (s, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.4, 156.1, 136.0, 128.4, 128.3, 127.9, 127.8, 127.7, 81.2, 81.1, 67.2, 67.0, 60.4, 59.9, 59.0, 55.2, 55.1, 38.6, 37.7, 28.9, 28.7, 27.8, 27.7. - Cis-isomer: $[\alpha]_D^{22}$ = -54.0 (c = 1.51, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$): δ= 1.33 [s, 9 H, $C(CH_3)_3$], 1.4-1.24 (m, 6 H, CH_2-CH_2, CH_2-CH_2-O), 3.6-3.9 (m, 2 H, CH_2OH), 4.08 (dd, J = 9.5, J = 4, 1 H, OH), 4.25 (dd, J = J 8.5, 1 H, $CHCO_2tBu$), 4.40 (m, 1 H, CH_2-CH-N), 5.15 (m, 2 H, CH_2Ph), 7.35 (s, 5 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$): 6 = 27.7, 28.9, 30.4, 37.4, 55.4, 58.8, 60.5, 67.4, 81.3, 127.7, 127.9, 128.3, 136.1, 155.9, 171.8.

[0173] A solution of the alcohol (0.304 g, 0.87 mmol) in dry CH_2Cl_2 (2.5 mL) was added to a suspension of Dess-Martin periodinane (0.408 g, 1.13 mmol) in dry CH_2Cl_2 (2.5 mL) at room temperature. After 1h Et_2O and NaOH 1N were added till clear solution. The aqueous phase was extracted twice with Et_2O; the collected organic layers were washed with H_2O, dried with Na_2SO_4, and evaporated to dryness. The crude product was purified by flash chromatography (hexane/ethyl acetate 7:3) affording 0.277 g of 17 (92%). - Trans-isomer: $[\alpha]_D^{22}$ = -48.65 (c = 1.01, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ= 1.35-1.45 [2 s, 9 H, $C(CH_3)_3$], 1.6-2.6 (m, 4 H, CH_2-CH_2), 2.8-3.1 (2 m, 2 H, CH_2CHO), 4.3 (m, 1 H, CHO-CH_2-CH-N), 4.6 (m, 1 H, N-CH-COOR), 5.15 (m, 2 H, CH_2Ph), 7.30 (m, 5 H, aromatic), 9.1, 9.3 (2 m, 1H, CHO). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 200.3, 171.4, 154.1, 136.2, 128.4, 128.2, 128.0, 127.8, 127.7, 81.3, 67.1, 66.9, 60.5, 60.1, 53.4, 52.5, 49.0, 48.4, 29.5, 28.6, 28.3, 27.8, 27.7, 27.3.

[0174] N-Boc-protected enamide (**44**): The mixture of aldehydes **14** and **17** was reacted following the general procedure A. The crude product was purified by flash chromatography (hexane/ethyl acetate 7:3), affording the enamide in 99% yield, as a trans:cis, Z/E mixture. Trans-Z-isomer : $[\alpha]_D^{22}$ = -61.84 (c = 1.01, $CHCl_3$). - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism) : δ = 1.35-1.50 [2 s, 9 H, $C(CH_3)_3$], 1.6-2.3 (m, 4 H, CH_2-CH_2), 2.3-2.8 (2 m, 2 H, =CH-CH_2), 3.75 (s, 3 H, $COOCH_3$), 4,15-4.25 (2 m, 2 H, -CH_2-CH-N and N-CH-COOtBu), 5.15 (m, 4 H, CH_2Ph), 6.55 (t, J = 8.5 Hz, 1 H, =CH), 7.35 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were

splitted for amidic isomerism): δ = 171.4, 164.8, 164.6, 154.4, 153.9, 153.7, 136.4, 136.2, 135.9, 135.7, 133.0, 132.0, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 126.7, 81.2, 67.3, 67.2, 67.0, 66.8, 60.6, 60.2, 57.6, 56.7, 52.3, 33.5, 32.5, 28.5, 27.7, 27.4. - FAB+MS: calcd. for $C_{30}H_{36}N_2O_8$ 552.6, found 552. -

[0175] Trans-E-isomer : $[\alpha]_D^{22}$ = -50.16 (c = 1.48, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.35-1.45 [2 s, 9 H, $C(CH_3)_3$] 1.6-2.4 (m, 4 H, CH_2-CH_2), 2.7-3.1 (2 m, 2 H, =CH-CH_2), 3.8 (2 s, 3 H, $COOCH_3$), 4,1-4.3 (2 m, 2 H, -CH_2-CH-N e N-CH-COOtBu), 5.10 (m, 4 H, CH_2Ph), 6.50 (m, 1 H, =CH), 7.25 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): 6 = 171.7, 171.5, 164.2, 164.0, 154.7, 154.3, 153.5, 136.6, 136.4, 135.8, 128.4, 128.3, 128.2, 128.1, 127.7, 126.2, 125.9, 125.8, 81.0, 87.1, 66.8, 66.6, 60.3, 60.4, 58.2, 57.3, 52.2, 32.8, 31.9, 28.5, 28.1, 27.8, 27.7, 27.4, 27.1.

[0176] The mixture of enamides (0.394 g, 0.71 mmol) was reacted following the general procedure B. Flash chromatography of the crude product (hexane/ethyl acetate 75:25) afforded 0.287 g (73%) of pure trans-isomer 23. - Z-isomer: $[\alpha]_D^{22}$ = -50.98 (c = 1.56, $CHCl_3$). - ^1H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.3-1.5 [4 s, 18 H, $C(CH_3)_3$], 1.7-2.6 (m, 6 H, CH_2-CH_2 and =CH-CH_2), 3.7 (s, 3 H, $COOCH_3$), 4,1-4.3 (m, 2 H, - CH_2-CH-N and N-CH-COOtBu), 5.15 (m, 4 H, CH_2Ph), 6.8 (m, 1 H, =CH), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 171.4, 163.9, 154.6, 154.5, 150.0, 146.2, 138.5, 138.0, 136.2, 129.9, 128.3, 128.2, 128.1, 127.8, 83.4, 81.2, 68.3, 67.0, 66.8, 60.6, 60.2, 56.9, 56.2, 52.2, 32.9, 32.0, 28.3, 27.8, 27.7, 27.3. - FAB+MS: calcd. for $C_{35}H_{44}N_2O_{10}$ 652.7, found 652. -

[0177] E-isomer: ^1H NMR (200 MHz, $CDCl_3$): δ = 1.3-1.4 [2 s, 18 H, $C(CH_3)_3$], 1.5-2.3 (m, 4 H, CH_2-CH_2), 3.0 (2 m, 2 H, =CH-CH_2), 3.65 (2 s, 3 H, $COOCH_3$), 4,2 (m, 2 H, -CH_2-CH-N and N-CH-COOtBu), 5.15 (m, 4 H, CH_2Ph), 6.1 (2 t, J = 8.5 Hz, 1 H, =CH), 7.30 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) : δ = 171.5, 163.7, 154.6, 154.3, 152.2, 150.4, 142.7, 142.2, 136.3, 135.1, 128.9, 128.3, 128.2, 128.0, 127.8, 127.7, 83.4, 83.3, 81.1, 77.1, 68.3, 66.9, 66.7, 60.7, 60.3, 57.6, 56.8, 51.7, 32.9, 32.0, 28.4, 28.0, 27.7, 27.3, 27.0.

Example 11

6,5 fused bicyclic lactams (**5a**, **11a**):

[0178] A solution of **44** (0.489 g, 0.75 mmol) and $Pd(OH)_2$/C 20% (catalytic) in MeOH (7.5 mL) was stirred under H_2 for one night. The catalyst was filtered off and the mixture was refluxed for 24h. The solvent was then removed and the two diastereoisomeric products were separated by flash chromatography (hexane/ethyl acetate 6:4), yielding 0.186 g of **5a** and **11a** (70%) in a 1.4:1 diastereoisomeric ratio. - **5a**: ^1H NMR (200MHz, $CDCl_3$): δ = 1.45-1.50 [2 s, 18 H, $C(CH_3)_3$], 1.55-2.60 (m, 8 H, CH_2-CH_2 and BocN-CH-CH_2-CH_2), 3.68 [tt, J = 14.9 Hz and 4.2 Hz, 1 H, $(R)_2CH$-N], 4.05 (m, 1 H, CH-NBoc), 4.35 (t, J = 8.5 Hz, 1H, N-CH-COOtBu), 5.28 (broad, 1 H, NH). - FAB+MS: calcd. for $C_{18}H_{32}N_2O_5$ 354.46, found 354. -

[0179] **11a**: $[\alpha]_D^{22}$ = -107.9 (c = 1.7, $CHCl_3$). - ^1H NMR (200MHz, $CDCl_3$): δ= 1.45-1.50 [2 s, 18 H, $C(CH_3)_3$], 1.75-2.50 (m, 8 H, CH_2-CH_2 and BocN-CH-CH_2-CH_2), 3.70 [m, 1 H, CH-N], 4.15 (m, 1 H, CH-NBoc), 4.50 (t, J = 7.0 Hz, 1H, N-CH-COOtBu), 5.55 (broad, 1 H, NH). - ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 170.6, 168.5, 155.5, 81.4, 79.3, 59.0, 56.2, 49.9, 32.3, 28.1, 27.8, 26.5, 25.9. - FAB+MS: calcd. for $C_{18}H_{32}N_2O_5$ 354.46, found 354.

Aldehyde (**18**):

[0180] The general procedure C was followed using **43** and the crude residue was purified by flash chromatography affording the alcohol with a yield of 98%. - ^1H NMR (200 MHz, $CDCl_3$) δ= 1.32 [s, 9 H, $C(CH_3)_3$], 1.4-2.4 (m, 8 H, CH_2-CH_2), 3.5-3.7 (m, 2 H, CH_2OH), 4.1 (m, 1 H, CH_2-CH-N), 4.24 (m, 1 H, N-CH-COOtBu), 5.05 (s, 2 H, CH_2Ph), 7.25 (m, 5 H, aromatic).

[0181] The general procedure D was followed using the alcohol and the crude was purified by flash chromatography (hexane/ethyl acetate 6:4) affording **18** with a yield of 82% - ^1H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ= 1.32, 1.45 [2 s, 9 H, $C(CH_3)_3$], 1.5-2.7 (m, 8 H, CH_2-CH_2), 4.1 (m, 1 H, CH_2-CH-N), 4.25 (m, 1 H, N-CH-COOR), 5.15 (s, 2 H, CH_2Ph), 7.20-7.40 (m, 5 H, aromatic), 9.6-9.8 (2 m, 1 H, CHO).

Enamide (**46**):

[0182] The general procedure A was followed using **18** and the crude was purified by flash chromatography (hexane/ethyl acetate 6:4) affording the enamide with a yield of 90% (diastereomeric ratio Z/E = 7:1) - ^1H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ= 1.32, 1.42 [s, 9 H, $C(CH_3)_3$], 1.5-2.7 (m, 8 H, CH_2-CH_2), 3.71 (s, 1 H, $COOCH_3$), 4.1 (m, 1 H, CH_2-CH-N), 4.22 (m, 1 H, N-CH-COOtBu), 5.0-5.20 (m, 4 H, CH_2Ph), 6.6 (m, 1 H, =CH), 7.20-7.45 (m, 10 H, aromatic).

[0183] The general procedure B was followed using the enamide and the crude residue was purified by flash chro-

matography yielding **46** (98%). - ^{1}H NMR (200 MHz, $CDCl_3$), (signals were splitted for amidic isomerism): δ= 1.32, 1.42 [2 s, 18 H, C(CH_3)$_3$], 1.5-2.2 (m, 8 H, CH_2-CH_2), 3.71 (s, 1 H, COOCH_3), 3.9 (m, 1 H, CH_2-C*H*-N), 4.22 (m, 1 H, N-CH-COOtBu), 5.0-5.20 (m, 4 H, CH_2Ph), 6.9 (m, 1 H, =C*H*), 7.20-7.45 (m, 10 H, aromatic). - ^{13}C NMR (50.3 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ= 141.6, 128.4, 128.2, 128.1, 127.8, 127.7, 68.2, 66.8, 60.5, 58.1, 52.1, 31.3, 29.5, 27.1, 27.3, 24.6.

Example 12

trans-7,5-fused bicyclic lactam (**6**a, **12**a):

[0184] To a solution of **46** (0.093 g, 0.141 mmol) in MeOH (2 ml) was added 1N NaOH (0.705 mmol, 0.705 ml) and stirred for 1.5 h. The solution was acidified until pH 3 with 1N HCl, then the solution was evaporated. The crude was submitted to the next reaction without further purification. - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ = 1.25, 1.48 [2 s, 18 H, C(CH_3)$_3$], 1.5-2.4 (m, 8 H, CH_2-CH_2), 4.1 (m, 1 H, CH_2-C*H*-N), 4.3 (m, 1 H, N-CH-COOtBu), 5.12 (s, 2 H, CH_2Ph), 6.65 (m, 1 H, =CH), 7.1-7.4 (m, 5 H, aromatic), 9.00 (bs, 1 H, -COO*H*).

[0185] A solution of previous compound in xylene was refluxed for 48 h. The solvent was evaporated and the crude was purified by flash chromatography yielding **6**a and **12**a with a 40% of yield.

[0186] **6**a - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ= 1.43, 1.45 [2 s, 18 H, C(CH_3)$_3$], 1.51-2 40 (m. 10 H. CH_2-CH_2), 3.75 [m, 1 H, CH-N], 4.22 (m, 1 H, C*H*-NBoc), 4.48 (t, J = 17 Hz, 1H, N-C*H*-COOtBu), 5.7 (broad. 1 H, N*H*).

[0187] **12**a - ^{1}H NMR (200 MHz, $CDCl_3$) (signals were splitted for amidic isomerism): δ= 1.47, 1.48 [2 s, 18 H, C (CH_3)$_3$], 1.55-2.50 (m, 8 H, CH_2-CH_2), 4.0 (m, 1 H, CH-N), 4.30 (m, 1 H, C*H*-NBoc), 4.50 (dd, J = 5.4 Hz, J = 17 Hz, 1H, N-C*H*-COOtBu), 6.0 (bd, 1 H, N*H*).

Example 13

[0188] Using the bicyclic lactams prepared according to the preceding examples, the respective peptidomimetics compounds, containing the RGD sequence were prepared according to the method disclosed in Gennari et al.: Eur. J. Org. Chem., 1999, 379-388.

[0189] Examples 14-47 may be read easier by making reference to Figures 9-12.

Example 14

[0190] Reagents and solvents: Sasrin resin (200-400 mesh, 1.02 mmol/g) was purchased from Bachem. All the solvents used for the solid-phase synthesis were of HPLC quality or Analyticai Reagent grade and were dried over molecular sieves before use. Flash chromatography: silica gel (Kieselgel 60, 230-400 mesh). TLC: silica plates (60 F_{254}, 0.25 mm, Merck). NMR: Bruker AC-200, AC-300 and Avance-400 (200 MHz, 300 MHz and 400 MHz for ^{1}H, 50.3 MHz, 75.4 MHz and 100.5 MHz for ^{13}C). Optical rotations: Perkin Elmer 241 polarimeter. Mass spectrometry: VG 7070 EQ-HF and PE-SCIEX API-100. Elemental analysis: Perkin Elmer 240. All solid-phase reaction were carried out on a wrist shaker.

[0191] Abbreviations: DCM: dichloromethane, DIC: N,N'-diisopropylcarbodiimide, HOAt 1-hydroxy-7-azabenzotriazole, HOBt: 1-hydroxybenzotriazole, HATU: 0-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, TNBS: 2,4,6-trinitrobenzenesulfonic acid.

[0192] TNBS test was performed following this procedure: a few resin beads were sampled and washed several times with ethanol. The sample was then placed in a vial and 1 drop of a 10 % solution of DEPEA in DMF and 1 drop of 1 % 2,4,6-trinitrobenzenesulfonic acid (TNBS) in DMF were added. The sample was then observed and colour changes were noted. The TNBS test is considered to be positive (presence of free amino groups) when the resin beads turn orange or red within 1 min and negative (no free amino groups) when the beads remain colourless.

[0193] General Procedure 1. Preparation of N-Fmoc-Temp-OH (Temp 1-8). To a solution of the starting N-Boc-Temp-OtBu (0.62 mmol) in dichloromethane (4.8 ml) was added, under N_2, trifluoroacetic acid (4.8 ml) and the resulting mixture was stirred at room temperature for 1 h. The solvents were then evaporated under reduced pressure, the crude residue was dissolved in THF (0.32 ml) and 10 % Na_2CO_3 (0.77 ml) was added. After 15 min the solution was cooled to 0°C, a solution of Fmoc-ONSu (95 mg) in THF (1.4 ml) was added and the resulting mixture was stirred at room temperature for 3 h (TLC $CHCl_3$/MeOH/AcOH 75:25:5). THF was then evaporated under reduced pressure, the aqueous phase was washed with AcOEt, conc. HCl was added to pH 3-4 and the solution extracted with AcOEt (3 x 5 ml). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to afford the crude product as a white fbam, which was used without further purification.

Example 15

(3S,6S,9S)-1-aza-9-carboxy-3-(9'-fluorenylmetohxycarbonylamino)-2-oxo-bicyclo[4.3.0] nonane (Temp1).

[0194] Was prepared in quantitative overall yield following general procedure 1. ^{1}H NMR (200 MHz, C_6D_6, 3 23°K) 6 = 1.1-2.0 (m, 8 H, 4 C*H*$_2$), 2.9 (m, 1 H, C*H*-N), 4.12 (dd, $J_1 = J_2 = 6.5$ Hz, 1 H, C*H*-CH_2O), 4.20-4.50 (m, 4 H, C*H*-NHFmoc, C*H*CO_2H, C*H*$_2$O) 6.30 (d, J = 7 Hz, 1 H, N*H*), 7.10-7.30 (m, 4 H, aromatic), 7.45-7.65 (m, 4 H, aromatic), 11.2 (bs, 1 H, CO_2*H*).

[0195] ^{13}C NMR (50.3 MHz, CDCl3) δ = 26.5, 26.9, 28.8, 31.9, 47.0, 50.2, 56.9, 58.5, 67.1, 119.8, 125.2, 127.1, 127.6, 141.2, 143.7, 143.9, 15 6.6, 170.3, 173.2, 174.0.

[0196] MS (FAB$^+$): 421 (M+1).

Example 16

(3R,6S,9S)-1-aza-9-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (Temp2).

[0197] Was prepared in quantitative overall yield following general procedure 1. ^{1}HNMR - (200 MHz, C_6D_6, 323°K) δ= 1. 1-2.0 (m, 8 H, 4 C*H*$_2$), 3.0 (m, 1 H, C*H*-N), 3.9 (m, 1 H, C*H*-NHFmoc), 4.12 (dd, J, = J2 = 6.5 Hz, 1 H, CH-$CH_2$0), 4.25-4.55 (m, 3 H, C*H*C02H, C*H*20), 6.02 (bs, 1 H, N*H*), 7.10-7.25 (m, 4 H, aromatic), 7.45-7.60 (m, 4 H, aromatic), 7.70 (bs, 1 H CO_2*H*), ^{13}C NMR (50.3 MHz, CDCl3) δ = 27.7, 27.8, 28.1, 31.3, 47.0, 51.8, 58.6, 60.5, 67.0, 119.8, 125.1, 127.0, 127.6, 141.1, 143.8, 156.5, 170.0, 173.2, 174.3.

[0198] MS (FAB+): 421 (M+1).

Example 17

(3S, 6R, 9S)-1-aza-9-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (Temp3).

[0199] Was prepared in quantitative overall yield following general procedure 1. ^{1}H-NMR (200 MHz, C_6D_6, 323°K) δ = 1.1-2.0 (m, 8 H, 4 C*H*$_2$), 3.0-3.2 (m, 1 H, C*H*-N), 4.20 (dd, $J_1 = J_2 = 7$ Hz, 1 H, C*H*-CH_2O), 4.25-4.50 (m, 4 H, CH-NHFmoc, C*H*CO_2H, C*H*$_2$O), 6.43 (d, J = 7 Hz, 1 H, N*H*), 7.10-7.30 (m, 4 H, aromatic), 7.40-7.80 (m, 4 H, aromatic), 10.80 (bs, 1 H, CO_2*H*). ^{13}C-NMR (50.3 MHz, CDC13) δ = 27.3, 27.4, 28.0, 32.5, 47.1, 51.9, 58.9, 60.2, 67.1, 119.8, 125.2, 127.0, 127.6, 141.2, 143.8, 143.9, 156.8, 169.5, 173.8. MS (FAB$^+$): 421 (M+1).

Example 18

(3R,6R,9S)-1-aza-9-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[4.3.0]nonane (Temp4).

[0200] Was prepared in quantitative overall yield following general procedure 1. ^{1}H-NMR (200 MHz, C_6D_6, 323°K) δ = 0.8-1.9 (m, 8 H, 4 C*H*$_2$), 3.15 (m, 1 H, C*H*-N), 4.10 (dd, $J_1 = J_2 = 6$ Hz, 1 H, C*H*-CH_2O), 4.30-4.60 (m, 4 H, C*H*-NHFmoc, C*H*CO_2H, C*H*$_2$O), 6.20 (bs, 1 H, NH), 7.10-7.30 (m, 4 H, aromatic), 7.50-7.60 (m, 4 H aromatic), 9.80 (bs, 1 H, CO_2*H*). ^{13}C-NMR (50.3 MHz, $CDCl_3$) δ = 25.3, 26.6, 27.5, 32.1, 46.9, 49.9, 57.7, 58.8, 67.2, 119.8, 125.1, 127.0, 127.6, 141.1, 143.7, 143.8, 156.6, 173.1, 174.2. MS (FAB$^+$): 421 (M+1).

Example 19

(3S,7S,10S)-1-aza-10-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[5.3.0]decane (Temp 5).

[0201] Was prepared in quantitative overall yield following general procedure 1. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1. 2-2.4 (m, 10 H, 5 C*H*$_2$), 2.72 (s, 1 H C*H*-CH_2O), 3.90 (m, 1 H, C*H*-N), 4.25 (m, 1 H, C*H*-CO_2H), 4.4 (m, 2 H, C*H*$_2$O), 4.75 (m, 1 H, CH-NHFmoc), 6.35 (d, J = 5 Hz, 1 H, N*H*), 7.3-7.9 (m, 8 H, aromatic), 9.6 (s, CO_2*H*). ^{13}C-NMR (50.3 MHz, CDCl3) δ = 25.36, 27.15, 27.39, 31.34, 32.97, 34.00, 47.20, 54.75, 59.39, 60.67, 67.08, 119.84, 125.07, 127.02, 127.59, 141.23, 143.84, 143.92, 155.78, 172.04, 174.67. MS (FAB$^+$): 435 (M+1).

Example 20

(3R;7S,10S)-1-aza-10-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[5.3.0]decane (Temp6).

[0202] Was prepared in quantitative overall yield following general procedure 1. ^{1}H-NMR (200 MHz, $CDCl_3$) δ =

1.6-2.3 (m, 10 H, 5 CH_2), 2.65 (s, 1 H, CH-CH_2O), 3.98 (m, 1 H, CH-N), 4.22 (m, 1 H, CH-CO_2H), 4.5 (m, 3 H, CH-NHFmoc, CH_20), 6.3 (bs, 1 H, NH), 7.28-7.85 (m, 8 H, aromatic), 9.6 (bs, 1 H, CO_2H). ^{13}C-NMR (50.3 MHz, $CDCl_3$) δ = 22.02, 25.25, 26.63, 32.96, 46.92, 58.54, 60.60, 61.76, 68.74, 119.91, 124.77, 124.82, 127.00, 127.7 1. MS (FAB$^+$): 435 (M+1).

Example 21

(3R,7R,10S)-1-aza-10-carboxy-3-(9'-fluorenylmethoxycarbonylamino)-2-oxo-bicyclo[5.3.0]decane (Temp7).

[0203] Was prepared in quantitative overall yield following general procedure 1. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.70-2.35 (m, 10 H, 5 CH_2), 2.70 (m, 1 H, CH-CH_2O), 4.05 (m, 1 H, CH-N), 4.25 (m, 1 H, CH-NHFmoc), 4.40 (m, 2 H, CH20),4.65 (m, 1 H, CH-CO_2H), 6.15 (bs, 1 H, NH), 7.10-7.80 (8 H, aromatic). ^{13}C-NMR (50.3 MHz, $CDCl_3$) δ = 25.3, 26.9, 29.6, 32.1, 34.0, 47.0, 54.7, 59.4, 60.4, 67.1, 119.8, 119.9, 124.6, 125.1, 127.0, 127.5, 127.6, 131.1. MS (FAB$^+$): 435 (M+1).

Example 22

General Procedure 2. Preparation of N-Fmoc-Gly-0-Sasrin Resin.

[0204] In a solid phase reaction vessel, Sasrin resin (500 mg, 0.51 mmol) was suspended in a solution of N-Fmoc-Gly-OH (455 mg, 1.53 mmol), HOBt (206 mg, 1.53 mmol), DIC (0.24 ml, 1.53 mmol) and DMAP (19 mg, 0.15 mmol) in DMF (10 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml) and DCM (3 x 10 ml). The possibly unreacted hydroxy groups present were capped by treatment with a solution of acetic anhydride (0.096 ml, 1 mmol) and DMAP (57 mg, 0.51 mmol) in DMF (12 ml) for 2 h. The solution was drained and the resin washed with DMF (3 x 10 ml) and DCM (3 x 10 ml).

Example 23

General Procedure 3. Preparation of N-Fmoc-Arg(Pmc)-Gly-O-Sasrin Resin.

[0205] In a solid phase reaction vessel, N-Fmoc-Gly-O-Sasrin resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. N-Fmoc-Arg (Pmc)-OH (1.014 g, 1.53 mmol) and HOAt (208 mg, 1.53 mmol) were dissolved in DCM/DMF 2:1 (10 ml). At 0°C, DIC (0.24 ml, 1.53 mmol) was added dropwise to this solution. The resulting mixture was stirred for 10 min at this temperature and for a further 10 min at room temperature, then added to the resin. This mixture was shaken at room temperature for 2.5 h. The solution was drained and the resin washed with DMF (3 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 ml) for 2 h. The solution was drained and the resin washed with DCM (3 x 10 ml).

Example 24

General Procedure 4. Preparation of N-Fmoc-Temp-Arg(Pmc)-Gly-O-Sasrin Resin.

[0206] In a solid phase reaction vessel, N-Fmoc-Arg(Pmc)-Gly-O-Sasrin resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. The resin was suspended in a solution of N-Fmoc-Temp-OH (0.54 mmol), HATU (387 mg, 1.02 mmol), HOAt (139 mg, 1.02 mmol) and 2,4,6-collidine (0.135 ml, 1.02 mmol) in DMF/DCM 3:1 (13 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 ml) for 2 h. The solution was drained and the resin was washed with DCM (3 x 10 ml).

Example 25

General Procedure 5. Preparation of N-Fmoc-Asp(tBu)-Temp-Arg(Pmc)-Gly-O-Sasrin Resin.

[0207] In a solid phase reaction vessel, N-Fmoc-Temp-Arg(Pmc)-Gly-O-Sasrin resin (0.51 mmol) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 Ml). The deprotection was assessed by performing a TNBS test. The resin was suspended in a solution of N-Fmoc-Asp(tBu)-OH (840 mg, 2.04 mmol), HATU (776 mg, 2.04mmol), HOAt (278 mg, 2.04 mmol) and 2,4,6-collidine (0.27 ml, 2.04 mmol) in DMF/DCM 3:1 (13 ml) for 15 h. The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The success of the coupling was assessed by performing a TNBS test. The unreacted amino groups possibly present were capped by treatment with a solution of acetylimidazole (560 mg, 5.1 mmol) in DCM (12 Ml) for 2 h. The solution was drained and the resin was washed with DCM (3 x 10 ml).

Example 26

General Procedure 6. Cleavage of H_2N-Asp(tBu)-Temp-Arg(Pmc)-Gly-OH (1-7) from the Resin.

[0208] In a solid phase reaction vessel, N-Fmoc-Asp(tBu)-Temp-Arg(Pmc)-Gly-O-Sasrin resin (739 mg) was treated with a 20 % piperidine/DMF solution (10 ml, 1 x 3 min, 2 x 17 min). The solution was drained and the resin was washed with DMF (3 x 10 ml), MeOH (2 x 10 ml) and DCM (3 x 10 ml). The deprotection was assessed by performing a TNBS test. The resin was treated with 1 % TFA/DCM solution (7.4 ml x 3 min). The filtrates were immediately neutralized with a 18 % pyridine/MeOH solution (0.89 ml). The fractions containing the product (TLC DCM/MeOH 8:2) were combined and concentrated under reduced pressure to yield a residue, which was purified from the pyridinium salts by size-exclusion chromatography (AMBERLITE XAD-2 resin, H20 then MeOH). Evaporation of the combined MeOH fractions containing the product afforded a yellow residue which was used in the successive reaction without further purification.

Example 27

[0209] H_2N-Asp(tBu)-Templ-Arg(Pmc)-Gly-OH (1). Was prepared from the corresponding template in 40 % overall yield following general procedures 2-6. ^{1}H NMR (300 MHz, CD_3OD) δ = 1.30, 1.32 [2 s, 6 H, $(CH_3)_2C$-O], 1.43 [s, 9 H $(CH_3)_3CO$], 1.70 (m, 2 H, *H*-γ, Arg), 1.79-1.98 (m, 2 H, *H*-Cβ Arg), 1.85 (m, 2 H, CH_2CH_2Ar), 1.9 (m, 2 H, H-C_4 Temp), 2.1 (m, 4 H, H-Cs Temp, H-C_7 Temp), 2.1 (s, 3 H CH_3Ar), 2.2 (m, 2 H, *H*-C_8 Temp), 2.4 (dd, J = 9, 17, 1 H, *H*-C_β Asp), 2.55, 2.57 (2s, 6 H, CH_3Ar), 2.65 (m, 3 H, *H*-C_β Asp, CH_2CH_2Ar), 3.25 (m, 2 H, *H*-C_δ Arg), 3.65 (m, 1 H, *H*-C_6 Temp), 3.71 (d, J = 17, 1 H, *H*-C_α Gly), 3.75-3.95 (m, 1 H, *H*-Cα Asp), 3.87 (m, 1 H, *H*-Cα Gly), 4.25 (m, 1 H, *H*-C_3 Temp), 4.4 (dd, J = 0.8, 1 H, *H*-C_9 Temp), 4.88 (m, 1 H, H-Cα Arg). ^{13}C-NMR (50.3 MHz, CD_3OD) δ = 12.3, 17.9, 19.0, 22.4, 24.2, 27.0, 28.4, 29.0, 30.7, 33.8, 38.1, 41.4, 42.2, 48.4. 49.2, 50.5, 52.7, 55.2, 55.8, 62.2, 62.3. MS (FAB$^+$): 849 (M+1).

Example 28

H_2N-Asp(tBu)-Temp2-Arg(Pmc)-Gly-OH (2).

[0210] Was prepared from the corresponding template in 40 % overall yield following general procedures 2-6. MS (FAB$^+$): 849 (M+1).

Example 29

[0211] H_2N-Asp(tBu)-Temp3-Arg(Pmc)-Gly-OH (3). Was prepared from the corresponding template in 55 % overall yield following general procedures 2-6. ^{1}H-NMR (300 MHz, CD_3OD) δ = 1.30 [2 s, 6 H, $(CH_3)_2C$-O], 1.46 [s, 9 H, $(CH_3)_3CO$], 1.6 (m, 2 H, H-C7 Temp), 1.70 (m, 2 H, H-Cγ Arg), 1.85 (m, 2 H, CH_2CH_2Ar), 1.95-2.2 (m, 2 H *H*-C4 Temp), 2.0 (m, 2 H, *H*-Cβ Arg), 2.1 (s, 3 H, CH_3Ar), 2.2 (m, 2 H, *H*-C_5 Temp), 2.4-2.6 (m, 2 H, *H*-Cβ Asp), 2.55-2.6 (2s, 6 H, CH_3Ar), 2.65 (m, 2 H, CH_2CH_2Ar), 2.9 (m, 2 H, H-C_8 Temp), 3.2 (m, 2 H, H-C_6 Arg), 3.6 (d, J = 18, 1 H, *H*-Cα Gly), 3.80 (m, 1 H, H-C_6 Temp), 4.0 (m, 1 H, *H*-C_3 Temp), 4.05 (d, J = 18, 1 H *H*-Cα Gly), 4.2 (dd, J = 6.6, 1 H, *H*-C_9 Temp), 4.4 (m, 1 H, *H*-Cα Arg), 4.45 (m, 1 H, *H*-Cα Asp). ^{13}C-NMR (50.3 MHz, CD_3OD) δ = 12.3, 17.9, 19.0, 22.4, 27.0, 28.3, 28.6, 29.6, 30.0, 33.8, 37.0, 41.5, 43.3, 52.7, 54.2, 62.2, 74.9, 83.8, 119.4, 136.5, 169.7, 170.6, 173.9, 174.3. MS (FAB$^+$): 849 (M+1).

Example 30

H_2N-Asp(tBu)-Temp4-Arg(Pmc)-Gly-OH (4).

[0212] Was prepared from the corresponding template in 30 % overall yield following general procedures 2-6. MS (FAB+): 850 (M+2).

Example 31

H_2N-Asp(tBu)-Temp5-Arg(Pmc)-Gly-OH (5).

[0213] Was prepared from the corresponding template in 67 % overall yield following general procedures 2-6. MS (FAB+): 863 (M+1).

Example 32

H_2N-Asp(tBu)-Temp6-Arg(Pmc)-Gly-OH (6).

[0214] Was prepared from the corresponding template in 54 % overall yield following general procedures 2-6. MS (FAB+): 863 (M+1).

Example 33

H_2N-Asp(tBu)-Temp7-Arg(Pmc)-Gly-OH (7).

[0215] Was prepared from the corresponding template in 63 % overall yield following general procedures 2-6. MS (FAB+): 863 (M+1).

Example 34

H_2N-Asp(tBu)-Temp8-Arg(Pmc)-Gly-OH (8).

[0216] Was prepared from the corresponding template in 50 % overall yield following general procedures 2-6. MS (FAB+): 863 (M+1).

Example 35

[0217] General Procedure 7. Preparation of Cyclo[-Temp-Arg(Pmc)-Gly-Asp(tBu)-] (9-15). The linear peptide (0. 18 mmol) was dissolved in DMF (45 ml) under N2. HATU (205 mg, 0. 54 mmol), HOAt (73 mg, 0.54 mmol) and 2,4,6-collidine (0.072 ml, 0.54 mmol) were added and the resulting mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in AcOEt. The organic phase was washed twice with 5 % $NaHCO_3$, dried with Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (DCM/MeOH from 95:5 to 9: 1) to afford side-chain protected cyclopeptide as a yellow foam.

Example 36

Cyclo[-Templ-Arg(Pmc)-Gly-Asp(tBu)-] (9).

[0218] Was prepared in 70 % yield following general procedure 7. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.25, 1.3 [2 s, 6 H (CH_3)$_2$C-O], 1.46 [s, 9 H, (CH_3)$_3$CO], 1.5 (m, 2 H, H-Cy Arg), 1.6 (m, 2 H, H-C_4 Temp), 1.8 (m, 4 H, CH_2CH_2Ar, *H*-C_5 Temp), 2.0 (m, 2 H, *H*-Cβ Arg), 2.1 (s, 3 H, CH_3Ar), 2.2 (m, 4 H, H-C_7 Temp, H-C_8 Temp), 2.52, 2.56 (2 s, 6 H, CH_3Ar), 2.6 (m, 3 H, CH_2CH_2Ar, *H*-Cβ Asp), 3.1 (dd, J = 5, 17.6, 1 H, *H*-Cβ Asp), 3.25 (m, 2 H, *H*-Cδ Arg), 3.55 (m, 1 H, *H*-C_6 Temp), 3.65 (dd, J = 6, 13.6, 1 H, *H*-Cα Gly), 3.85 (dd, J = 4, 13.6, 1 H, *H*-Cα Gly), 4.22 (dd, J = 0, 9, 1 H *H*-C_9 Temp), 4.45 (m, 1 H, *H*-C_3 Temp), 4.54 (m, 1 H, *H*-Cα Arg), 4.68 (m, 1 H, *H*-Cα Asp), 6.3 (s, 3 H, *H*-Nε Arg, *H*NSO_2, = N*H*), 6.8 (d, J = 6, 1 H, N*H* Temp), 7.61 (d, J = 9, 1 N*H* Asp), 7.8 (d, J = 8, 1 H, N*H* Arg), 8.9 (bs, 1 H, N*H* Gly). ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ = 12.1, 17.4, 18.5, 21.4, 25.2, 26.2, 26.8, 27.5, 28.0, 29.6, 31.4, 32.2, 32.9, 36.4, 40.2, 46.0, 47.7, 50.3, 51.9, 60.6, 62.1, 73.5, 81.8, 117.8, 123.8, 134.8, 134.9, 135.5, 153.3, 156.5, 168.0, 169.8, 170.2, 170.9,

173.6, 175.0. $[\alpha]_D^{20}$ = -36.1 (c = 1.0, $CHCl_3$). MS (FAB^+): 830 (M^+), 853 (M+Na).

Example 37

Cyclo[-Temp3-Arg(Pmc)-Gly-Asp(tBu)-] (10).

[0219] Was prepared in 60 % yield following general procedure 7. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.3 [2 s, 6 H, $(CH_3)_2$C-O], 1.45 [s, 9 H, $(CH_3)_3CO$], 1.5 (m, 4 H, H-Cγ Arg, H-C_7 Temp), 1.7 (m, 1 H, *H*-Cβ Arg), 1.8 (m, 3 H, CH_2CH_2Ar, *H*-C_8 Temp), 1.9 (m, 1 H, H-C_4 Temp), 2.0 (m, 1 H, *H*-Cβ Arg), 2.05 (s, 3 H CH_3Ar), 2.2 (m, 3 H, *H*-C_4 Temp, *H*-C_5 Temp), 2.50 (m, 2 H, *H*-Cβ Asp, *H*-C_8 Temp), 2.52, 2.56 (2 s, 6 H, CH_3Ar), 2.6 (m, 2 H, CH_2CH_2Ar), 2.80 (dd, J = 8, 16, 1 H, *H*-Cβ Asp), 3.25 (m, 2 H, *H*-Cδ Arg), 3.45 (dd, J = 5, 16, 1 H, *H*-Cα Gly), 3.80 (m, 1 H, H-C_6 Temp), 4.10 (m, 1 H, *H*-C_3 Temp), 4.15 (m, 1 H, *H*-Cα Gly), 4.35 (dd, J= 10, 10, 1 H, *H*-C_9 Temp), 4.5 (m, 1 H, H-Cα Arg), 4.70 (m, 1 H, H-Ca Asp), 6.22 (bs, 3 H, *H*-Nε Arg, *H*NSO_2, =N*H*), 7.1 (d, J = 8, 1 H, N*H* Arg), 7.20 (d, J = 8, 1 H, N*H* Asp), 7.70 (d, J = 8, 1 H, N*H* Temp), 8.1 (bs, 1 H, N*H* Gly). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.4, 19.4, 21.3, 25.3, 26.7, 27.4, 27.9, 28.6, 29.6, 32.8, 36.7, 40.4, 45.1, 50.2, 50.7, 51.9, 60.7, 61.6, 73.5, 81.6, 117.8, 123.8, 133.7, 134.7, 135.3, 153.3, 156.3, 168.7, 169.8, 170.7, 171.4, 173.3. $[\alpha]_D^{20}$ = -57.1 (c = 1.0, $CHCl_3$). MS (FAB^+): 832 (M+2).

Example 38

Cyclo[-Temp4-Arg(Pmc)-Gly-Asp(tBu)-] (11).

[0220] Was prepared in 40 % yield following general procedure 7. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.3 [2 s, 6 H $(CH_3)_2$C-O] 1.4 (m, 1 H, *H*-C_5 Temp), 1.45 [s, 9 H $(CH_3)_3CO$], 1.5-1.6 (m, 4 H, *H*-Cγ Arg, *H*-C_4 Temp, *H*-C_8 Temp), 1.8 (m, 2 H, CH_2CH_2Ar), 1.97 (m, 1 H, *H*-C_8 Temp), 2.0 (m, 4 H, *H*-Cβ Arg, H-C_4 Temp, H-C_5 Temp), 2.15 (s, 3 H, CH_3Ar), 2.17, 2.43 (m, 2 H, *H*-C_7 Temp), 2.5 (m, 1 H, *H*-Cβ Asp), 2.60 (s, 3 H, CH_3Ar), 2.60 (m, 2 H, CH_2CH_2Ar), 2.62 (s, 3 H, CH_3Ar), 2.9 (dd, J = 7, 17, 1 H, *H*-Cβ Asp), 3.2 (m, 2 H, H-Cδ Arg), 3.55 (dd, J = 0, 12, 1 H *H*-Cα Gly), 4.05 (m, 1 H, *H*-C_9 Temp), 4.1 (m, 1 H, H-Cα Gly), 4.2 (m, 1 H, H-C_6 Temp), 4.3 (m, 1 H, H-C_3 Temp), 4.6 (m, 1 H, H-Cα Arg), 4.65 (m, 1 H, H-Cα Asp), 6.2-6.4 (bs, 3 H H-Nε Arg, *H*NSO_2, =N*H*), 7.3 (bs, 1 H, N*H* Temp), 7.45 (bs, 1 H NH Arg), 7.90 (bs, 1 H, N*H* Gly), 8.0 (bs, 1 H N*H* Asp). ^{13}C-NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.4, 18.4, 21.3, 22.0, 25.6, 26.2, 26.7, 27.9, 30.1, 32.7, 34.0, 35.0, 50.9, 51.0, 51.8, 56.5, 62.5, 73.5, 81.2, 95.0, 117.8, 123.9, 133.3, 134.7, 135.3, 153.4, 156.2, 170.4, 170.7, 171.9, 172.5, 173.3. $[\alpha]_D^{20}$ = -71.0 (c = 0.7, $CHCl_3$). MS (FAB^+): 832 (M+2).

Example 39

Cyclo[-Temp5-Arg(Pmc)-Gly-Asp(tBu)-] (12).

[0221] Was prepared in 35 % yield following general procedure 7. ^{1}H-NMR (300 MHz, DMSO-D_6) δ = 1.25, 1.38 [2 s, 6 H $(CH_3)_2CO$] 1.31 (m, 2 H, *H*-Cγ Arg), 1.38 [s, 9 H, $(CH_3)_3CO$], 1.8 (m, 2 H, CH_2CH_2Ar), 2.05 (s, 3 H, CH_3Ar), 2.05 (m, 1 H, *H*-C_9 Temp), 2,15 (m, 2 H, *H*-Cβ Arg), 2.35 (dd, J= 6.8, 17, 1 H *H*-Cβ Asp), 2.50,2,52 (2 s, 6 H, 3 CH_3Ar), 2.5 (m, 1 H, *H*-C_9 Temp), 2.6 (m, 2 H, CH_2CH_2Ar), 2.8 (dd, J = 8.6, 17, 1 H, *H*-Cβ Asp), 3.1 (m, 2 H, H-Cδ Arg), 3.61 (d, J = 9.8, 1 H, *H*-Cα Gly), 3.98 (d, J = 9.8, 1 H, H-Cα Gly), 4.0 (m, 2 H, H-Cα Arg, *H*-C_7 Temp), 4.31 (m, 2 H, *H*-C_3 Temp, *H*-C_{10} Temp), 4.55 (m, 1 H, H-Cα Asp), 6.48 (bs, 2 H, H-Nε Arg, $HNSO_2$), 6.78 (bs, 1 H, =N*H*), 7.68 (d, J = 5.1, 1 H, N*H* Temp), 7.84 (bd, 1 N*H* Asp), 8.22 (m, 1 H, NH Arg), 8.5 (bt, 1 H, NH Gly). ^{13}C-NMR (50.3 MHz, DMSO-D_6) δ=11.9, 17.1, 18.2, 26.4, 27.7, 20.8, 25.3, 27.0, 30.6, 32.2, 32.5, 36.3, 38.7, 40.3, 42.4, 49.6, 53.1, 58.8, 62.0, 73.5, 80.3. $[\alpha]_D^{20}$ =-36.7 (c = 1, $CHCl_3$). MS (FAB^+): 844 (M+).

Example 40

Cyclo[-Temp6-Arg(Pmc)-Gly-Asp(tBu)-] (13). Was prepared in 26 % yield following general procedure 7.

[0222] ^{1}H-NMR (300 MHz, DMSO-D_6) δ = 1.3 [s, 3 H $(CH_3)_2$C-O], 1.31 (m, 2 H, *H*-Cγ Arg), 1.38 [s, 9 H, $(CH_3)_3CO$], 1.4 [s, 3 H $(CH_3)_2$C-O], 1.8-1.95 (m, 6 H, CH_2CH_2Ar, *H*-C_9 Temp, *H*-Cβ Arg), 2.05 (s, 3 H CH_3Ar), 2.39 (dd, J = 4.3, 10.6, 1 H *H*-Cβ Asp), 2.50, 2,52 (2 s, 6 H, 3 CH_3Ar), 2.6 (m, 2 H, CH_2CH_2Ar), 2.9 (dd, J = 4.3, 10.6, 1 H *H*-Cβ Asp), 3.1 (m, 2 H *H*-Cδ Arg), 3.80 (m, 3 H, H-Cα Gly, *H*-Cα Arg), 4.3 (m, 1 H, *H*-C_3 Temp), 4.35 (m, 1 H, *H*-C_{10} Temp), 4.48 (m, 1 H, *H*-Cα Asp), 6.45 (bs, 2 H, H-Nε Arg, $HNSO_2$), 6.60 (bs, 1 H, =NH), 7.5 (bd, 1 H, N*H* Asp), 7.51 (bd, 1 H, NH Temp), 8.55 (m, 1 H, N*H* Arg), 8.75 (bt, 1 H, N*H* Gly). ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ = 12.1, 14.3, 17.5, 18.6, 21.4, 23.5, 26.8, 28.1, 29.7, 31.7, 32.8, 33.6, 49.7, 60.8, 73.6, 117.9, 124.0, 134.8, 135.5, 153.6, 171.5. $[\alpha]_D^{20}$ = -72.9 (c = 1, $CHCl_3$), MS (FAB^+): 844 (M+).

Example 41

Cyclo[-Temp7-Arg(Pmc)-Gly-Asp(tBu)-] (14). Was prepared in 15 % yield following general procedure 7.

[0223] ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.30 [s, 6 H, $(CH_3)_2C$-O], 1.41 [s, 9 H, $(CH_3)_3CO$], 1.49 (m, 1 H, *H*-Cγ Arg), 1.50-1.90 (10 H, CH_2CH_2Ar, H-C_5 Temp, H-C_6 Temp, H-C_5 Temp), 1.51 (m, 2 H, H-C_4 Temp), 1.60 (m, 1 H, *H*-Cγ Arg), 1.90 (m, 2 H, *H*-Cβ Arg), 1.98 (m, 1 H, *H*-C_9 Temp), 2.15 (s, 3 H CH_3Ar), 2.53 (s, 3 H, CH_3Ar), 2.58 (s, 3 H, CH_3Ar), 2.32 (m, 1 H, *H*-C_9 Temp), 2.51 (m. 1 H, *H*-Cβ Asp), 2.85 (m, 1 H, *H*-Cβ Asp), 3.20 (m, 2 H, *H*-Cδ Arg), 3.51 (bd, 1 H, *H*-Cα, Gly), 4.12 (m, 1 H, H-C_7 Temp), 4.18 (m, 1 H, H-Cα Gly), 4.32 (m, 1 H, H-C_{10} Temp), 4.5 (m, 1 H, H-C_3 Temp), 4.58 (m, 1 H, H-Cα Arg), 4.80 (m, 1 H, *H*-Cα Asp), 6.3 (bs, 3 H, *H*-Nε Arg, *H*NSO_2, =N*H*), 7.2 (bd, 1 H, NH Arg), 7.65 (bd, 1 H, NH Temp), 7.80 (bt, 1 H, NH Gly), 7.95 (bd, 1 H, N*H* Asp).

Example 42

[0224] Cyclo[-Temp8-Arg(Pmc)-Gly-Asp(tBu)-] (15). Was prepared in 55 % yield following general procedure 7. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.27, 1.31 [2 s, 6 R $(CH_3)_2C$-O], 1.44 [s, 9 H, $(CH_3)_3CO$], 1.50 (m, 3 H, *H*-Cγ Arg, *H*-C_6 Temp), 1.60 (m, 2 H, *H*-Cβ Arg, *H*-Cγ Arg), 1.70 (m, 2 H, *H*-C_8 Temp), 1.8 (m, 2 H, CH_2CH_2Ar), 1.85 (m, 1 H, *H*-C_4 Temp), 1.95 (m, 2 H, *H*-Cβ Arg, H-C_4 Temp), 1.98 (m, 2 H, H-C_5 Temp), 2.11 (s, 3 H, CH_3Ar), 2.32 (m, 1 H, *H*-C_9 Temp), 2.56 (s, 3 H CH_3Ar), 2.57 (dd, J = 7.4, 16.7, 1 H, *H*-Cβ Asp), 2.58 (s, 3 H CH_3Ar), 2.65 (m, 2 H, CH_2CH_2Ar), 2.87 (dd, J = 7.4, 16.7, 1 H, *H*-Cβ Asp), 3.20 (m, 2 H, *H*-C_3 Arg), 3.54 (bd, 1 H, *H*-Cα Gly), 4.18 (m, 1 H, *H*-Cα Gly), 4.22 (m, 1 H, H-C_7 Temp), 4.36 (m, 1 H, H-C_{10} Temp), 4.55 (m, 1H, H-C_3 Temp), 4.6 (m, 1 H, H-Cα Arg), 4.83 (m, 1 H, *H*-Cα Asp), 6.33 (bs, 3 H, *H*-Nε Arg, *H*NSO_2, =N*H*), 7.49 (bd, 1 H, N*H* Arg), 7.71 (bt, 1 H, N*H* Gly), 7.80 (bd, 1 H, N*H* Temp), 7.95 (bd, 1 H, NH Asp). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 12.0, 17.4, 18.4, 26.7, 27.4, 36.4, 21.4, 25.3, 28.5, 29.6, 30.8, 33.0, 34.9, 40.6, 44.3, 49.9, 51.9, 54.1, 59.3, 63.2, 73.5, 81.3, 117.8, 123.9, 133.4, 134.7, 135.3, 153.5, 156.3, 170.3, 170.5, 172.3, 172.6. $[\alpha]_D^{20}$ = -54 (c = 0.05, $CHCl_3$). MS (FAB$^+$): 844 (M+).

Example 43

General Procedure 8. Preparation of Cyclo(-Temp-Arg-Gly-Asp-) (16-22).

[0225] Side-chain protected cyclopeptide (0.1 mmol) was treated with TFA/thioanisole/ 1,2-ethanedithiol/anisole 90: 53:2 (35 ml) for 2 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in H20. The aqueous phase was washed twice with iPr20 and evaporated under reduced pressure to afford side-chain deprotected cyclopeptide as a white foam. Trifluoroacetate ion was substituted with chloride by ion-exchange chromatography (AMBERLITE IRA-93 resin, chloride form).

Example 44

[0226] Cyclo(-Templ-Arg-Gly-Asp-) (16). Was prepared in quantitative yield following general procedure 8. ^{1}H-NMR (400 MHz, D_2O) δ = 1.6-1.75 (m, 2 H, *H*-Cγ Arg), 1.65-1.95 (m, 6 H, *H*-C_4 Temp, *H*-C_5 Temp, *H*-C_7 Temp), 2.2 (m, 2 H, *H*-Cβ Arg), 2.3-2.45 (m, 2 H, *H*-C_8 Temp), 2.73 (dd, J= 0, 6, 2 H, *H*-Cβ Asp), 3.25-3.50 (m, 2 H, *H*-Cδ Arg), 3.8-3.95 (m, 1 H, H-C_6 Temp), 3.82 (d, J=13.5, 1 H, H-Cα Gly), 4.25 (d, J = 13.5, 1 H, *H*-Cα Gly), 4.50 (dd, J = 0, 10, 1 H, *H*-C_9 Temp), 4.55 (dd, J= 0, 8, 1 H, H-Cα, Arg), 4.58 (m, 1 H, H-C_3 Temp), 4.75 (m, 1 H, *H*-Cα Asp). ^{13}C NMR (75.4 MHz, D_2O) δ = 25.0, 26.4, 28.2, 29.8, 30.8, 32.2, 39.0, 41.3, 45.8, 48.3, 52.7, 53.1, 61.7, 62.6, 157.7, 170.1, 172.6, 174.0, 175.4, 175.7, 178.4. $[\alpha]_D^{20}$ = -52.6 (c = 0.88, H_2O). MS (FAB+: 509 (M+1)

Example 45

[0227] Cyclo(-Temp3-Arg-Gly-Asp-) (17). Was prepared in quantitative yield following general procedure 8. ^{1}H-NMR (400 MHz, D_2O) δ= 1.5 (m, 2 H, *H*-C_5 Temp), 1.6 (m, 2 H, *H*-Cβ Arg), 1.8 (m, 2 H, H-C_4 Temp), 1.9 (m, 2 H, *H*-Cγ Arg), 2.2 (m, 2 H, H-C_7 Temp), 2.42-2.52 (m, 2 H, *H*-C_8 Temp), 2.7-2.85 (m, 2 H, *H*-Cβ Asp), 3.15-3.30 (m, 2 H, *H*-Cδ Arg), 3.55 (d, J = 14, 1 H, *H*-Cα Gly), 3.83-3.95 (m, 1 H, *H*-C_6 Temp), 4.10 (d, J = 14, 1 H, H-Cα Gly), 4.28 (m, 1 H, H-C_3 Temp), 4.37 (dd, J = 0, 9, 1 H, *H*-C_8 Temp), 4.45 (dd, J = 5, 10, 1 H, *H*-Cα Arg), 4.65 (m, 1 H, H-Cα Asp). ^{13}C-NMR (50.3 MHz, D_2O) δ = 27.1, 29.3, 30.4, 31.4, 31.8, 35.1, 38.1, 43.3, 47.2, 52.8, 53.5, 54.8, 64.0, 64.5, 159.6, 166.1, 172.5, 174.8, 175.2, 176.4, 176.6, 177.9. $[\alpha]_D^{20}$ = -94.6 (c = 1.32, H_2O) MS (IS$^+$): 508 (M+).

Example 46

[0228] Cyclo(-Temp4-Arg-Gly-Asp-) (18). Was prepared in quantitative yield following general procedure 8. This compound was not stable in aqueous solution over a few days at room temperature. ^{1}H-NMR (400 MHz, D20) δ = 1.6-1.7 (m, 2 H, *H*-C_4 Temp), 1.7 (m, 2 H, *H*-Cγ Arg), 2.0 (m, 2 H, H-C7 Temp), 2.2 (m, 2 H, *H*-Cβ Arg), 2.4 (m, 2 H, *H*-C_5 Temp), 2.6 (m, 2 H, *H*-C_8 Temp), 2.70 (dd, J = 7, 17, 1 H, *H*-Cβ Asp), 3.05 (dd, J = 7, 17, 1 H, *H*-Cβ Asp), 3.15-3.25 (m, 2 H, *H*-C_8 Arg), 3.52 (d, J = 15, 1 H, *H*-Cα Gly), 4.05 (m, 1 H; *H*-C_6 Temp), 4.28 (d, J = 15, 1 H, H-Cα Gly), 4.3 (m, 1 H, *H*-C_3 Temp), 4.35 (m, 1 H, H-C_{10} Temp), 4.53 (dd, J = 7, 7, *H*-Cα Asp), 4.6 (m, 1H; *H*-Cα Arg). $[\alpha]_D^{20}$ = -63.7 (c = 0.95, H_2O). MS (IS$^+$): 508 (M+).

Example 47

[0229] Cyclo(-Temp5-Arg-Gly-Asp-) (19). Was prepared in quantitative yield following general procedure 8. ^{1}H-NMR (400 MHz, D_2O) δ = 1.5-1.8 (m, 2 H, *H*-Cγ Arg), 1.7-2.0 (m, 2 H, *H*-Cβ Arg), 2.8 (m, 2 H, *H*-Cβ Asp), 3.22 (m, 2 H, *H*-Cδ Arg), 4. 0 (m, 2 H, *H*-Cα Gly, *H*-C_7 Temp), 4.3 (dd, J = 7, 7, 1 H, *H*-Cα Arg), 4.5-4.6 (m, 1 H, *H*-C_3 Temp, *H*-C_{10} Temp), 4.68 (m, 1 H *H*-Cα Asp). ^{13}C-NMR (50.3 MHz, D2O) δ = 27.2, 29.8, 30.1, 31.0, 33.6, 35.3, 36.2, 39.0, 43.3, 45.7, 53.8, 56.3, 62.8, 65.2, 159.5, 174.3, 174.4, 175.6, 176.4, 178.5. $[\alpha]_D^{20}$ = -87.4 (c = 1.2, H_2O). MS (IS$^+$): 522 (M+).

Example 48

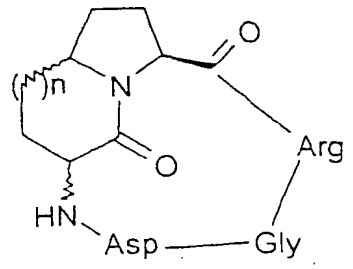
[0230] Cyclo(-Temp6-Arg-Gly-Asp-) (20). Was prepared in quantitative yield following general procedure 8. ^{1}H-NMR (400 MHz, D_2O) δ = 1,5-1.8 (m, 2 H, *H*-C_6 Temp), 1.6 (m, 2 H, *H*-Cγ Arg), 1.75-1.9 (m, 2 H, *H*-Cβ Arg), 1.8-1.95 (m, 2 H, *H*-C_4 Temp), 2.15 (m, 4 H, *H*-C_8 Temp, *H*-C_9 Temp), 2.65-2.8 (m, 2 H, *H*-Cβ Asp), 3.2 (m, 2 H, *H*-Cδ Arg), 3.82 (d, J = 17, 1 H, *H*-Cα Gly), 4.05 (d, J = 17, 1 H H-Cα Gly), 4.1 (m, 1 H, *H*-C_7 Temp), 4.37 (dd, J = 0, 7, 1 H, *H*-C_{10} Temp), 4.42 (dd, J = 0, 10, 1 H, *H*-C_3 Temp), 4.52 (dd, J = 5, 10, 1 H, *H*-Cα Arg), 4.70 (m, 1 H, *H*- Cα Asp). ^{13}C-NMR (75.4 MHz, D2O) δ = 22.3, 25.0, 25.9, 28.7, 30.4, 33.7, 34.2, 37.7, 41.4, 43.2, 51.5, 53.3, 57.5, 59.1, 63.6, 157.6, 171.6, 173.7, 174.2, 175.0, 176.9. $[\alpha]_D^{20}$ = -47.9 (c = 0.71, H_2O). MS (IS$^+$): 522 (M+).

Example 49

[0231] Cyclo(-Temp8-Arg-Gly-Asp-) (22). Was prepared in quantitative yield following general procedure 8. ^{1}H-NMR (400 MHz, D_2O) δ = 1.4 (m, 3 H, *H*-C_5 Temp, *H*-C_8 Temp), 1.55-1.7 (m, 2H, *H*-Cγ Arg), 1.8 (m, 4 H, H-C_4 Temp, *H*-C_8 Temp, *H*-C_9 Temp), 2.0 (m, 2 H, *H*-Cβ Arg), 2.26 (m, 2 H, *H*-C_6 Temp), 2.38 (m, 1 H, *H*-C_9 Temp), 2.68 (dd, J = 7, 18, 1 H, *H*-Cα Asp), 2.98 (dd, J = 7,18, 1 H, *H*-Cα Asp), 3.2 (m, 2 H, *H*-Cγ Arg), 3.5 (d, J = 15, 1 H, *H*-Cα Gly), 4.2 (d, J = 15, 1 H, *H*-Cα Gly), 4.2 (m, 1 H, *H*-C_7 Temp), 4.38 (m, 1 H, *H*-C_{10} Temp), 4.48 (dd, J = 5, 11, 1 H, *H*-Cα Arg), 4.53 (dd, J = 0, 11, 1 H, *H*-C_3 Temp), 4.63 (dd, J = 7, 7, 2 H, *H*-Cβ Asp). ^{13}C-NMR (75.4 MHz, D2O) δ = 27.4, 29.3, 30.2, 30.6, 33.0, 35.1, 36.2, 41.3, 46.1, 53.8, 54.9, 56.8, 62.8, 66.1, 159.5, 174.2, 174.8, 175.9, 176.4, 177.6. $[\alpha]_D^{20}$ = -38.1 (c = 1.2, H_2O). MS (IS$^+$): 522 (M+).

Claims

1. Compounds of formula (I)

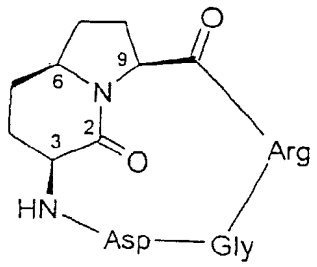


(I)

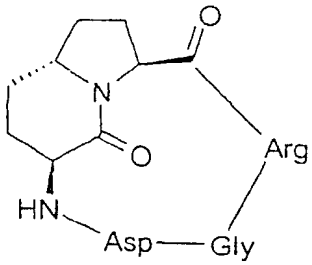
wherein n is the number 0, 1 or 2,

Arg is the amino acid L-Arginine, Gly is the amino acid Glycine and Asp is the amino acid L-Aspartic acid and the pharmaceutically acceptable salts thereof, their racemates, single enantiomers and diastereoisomers.

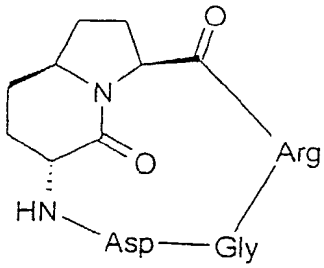
2. A compound of claim 1 which is



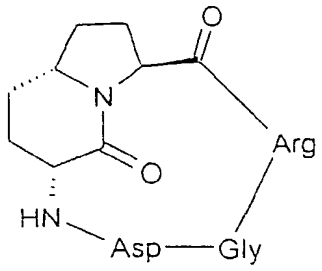
3. A compound of claim 1, which is



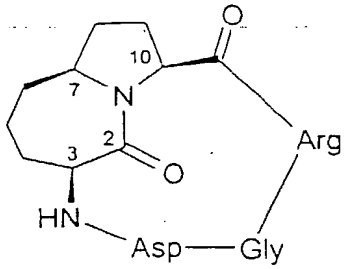
4. A compound of claim 1, which is



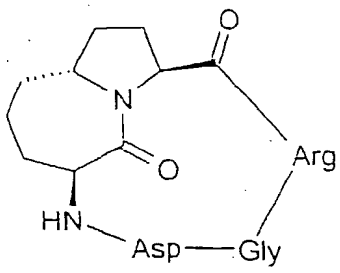
5. A compound of claim 1, which is



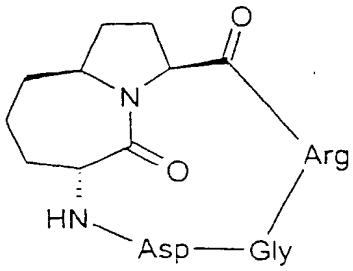
6. A compound of claim 1, which is



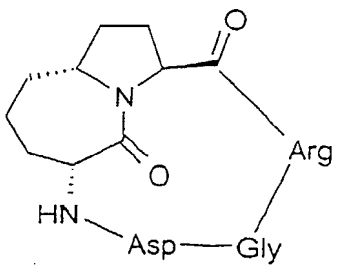
7. A compound of claim 1, which is



8. A compound of claim 1, which is

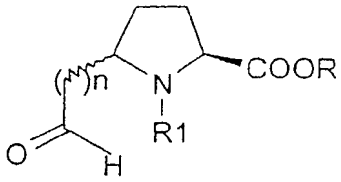


9. A compound of claim 1, which is



10. A process for the preparation of the compounds of claim 1 comprising the following steps:

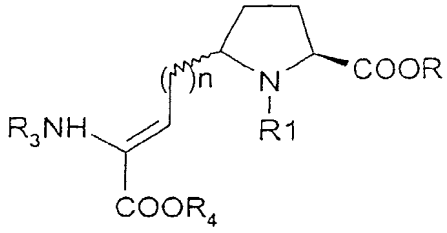
a) Horner-Emmons olefination of a compound of formula (II)



29) (II)

wherein

R is a lower alkyl residue;
R_1 is a suitable nitrogen protecting group, to give a compound of formula (III);



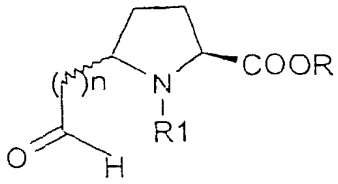
(III)

wherein R_3 is a suitable nitrogen protecting group, R_4 is a lower alkyl residue;

b) hydrogenation of said compound of formula (III) and cyclisation; and, if desired
c) separation of the stereoisomeric mixture;
d) building of the RGD cyclic sequence; and, if desired
e) separation of the stereoisomeric mixture.

11. A process for the stereoselective synthesis of the compounds of claim 1, comprising the following steps:

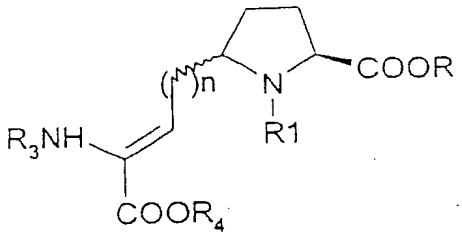
a) Horner-Emmons olefination of a compound of formula (II)



(II)

wherein

R is a lower alkyl residue;
R_1 is a suitable nitrogen protecting group, to give a compound of formula (III);



(III)

wherein R_3 is a suitable nitrogen protecting group, R_4 is a lower alkyl residue;

b) hydrogenation of said compound of formula (III) by chiral phosphine-Rh catalysed hydrogenation and cyclisation; and, if desired
c) separation of the stereoisomeric mixture;
d) building of the RGD cyclic sequence; and, if desired
e) separation of the stereoisomeric mixture.

12. Pharmaceutical composition comprising a therapeutically or preventive effective dose of at least a compound of claim 1 in admixture with pharmaceutically acceptable vehicles and/or excipients.

13. A method for selectively inhibiting $\alpha_v\beta_3$ integrin-mediated cell attachment to an RGD-containing ligand, comprising contacting said ligand with an effective amount of a compound of claim 1.

14. A method for treating a subject suffering from a pathology related to an altered $\alpha_v\beta_3$ integrin-mediated cell attachment comprising administering to said subject a compound of claim 1.

15. A method according to claim 14, wherein said pathology is retinopathy.

16. A method according to claim 14, wherein said pathology is acute renal failure.

17. A method according to claim 14, wherein said pathology is osteoporosis.

18. A method for treating a subject suffering from altered angiogenesis, comprising administering to said subject a compound of claim 1.

19. A method for the treatment of tumors in a subject comprising administering to said subject a compound of claim 1.

20. A method according to claim 19, wherein said tumor, is associated with metastasis.

FIGURE 1

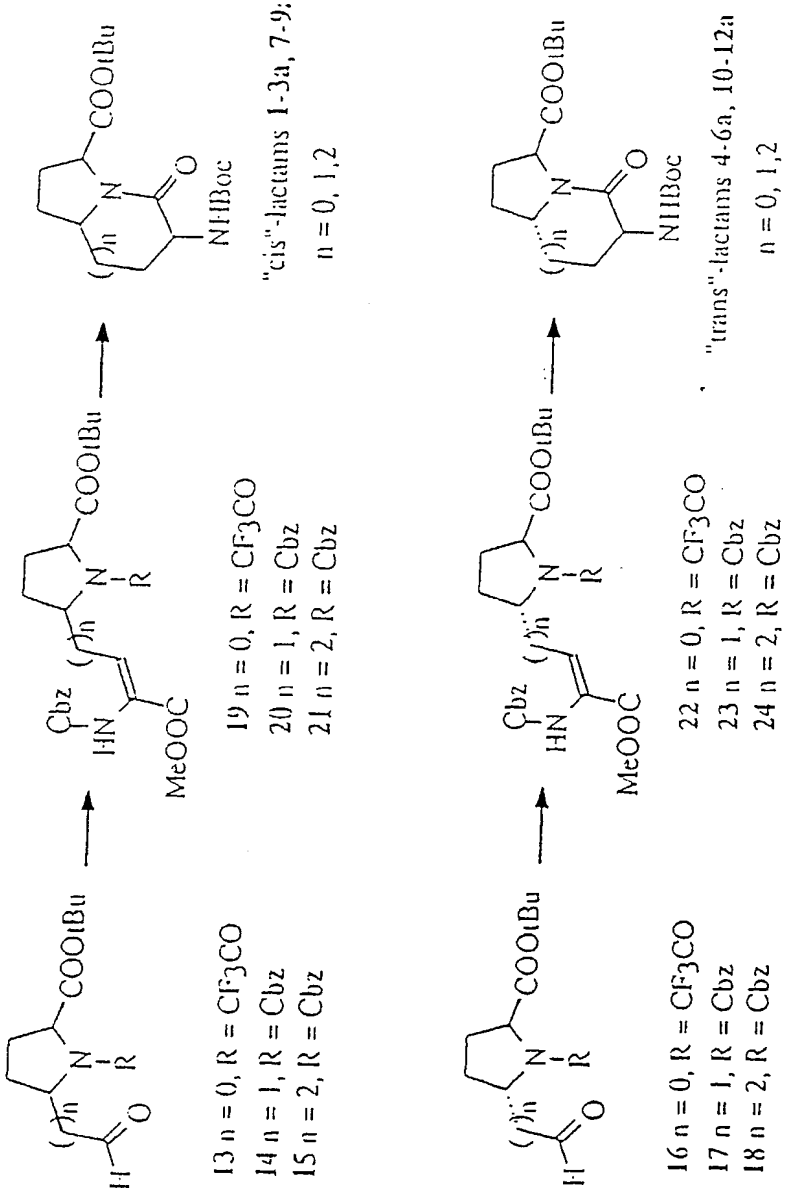


Figure 2

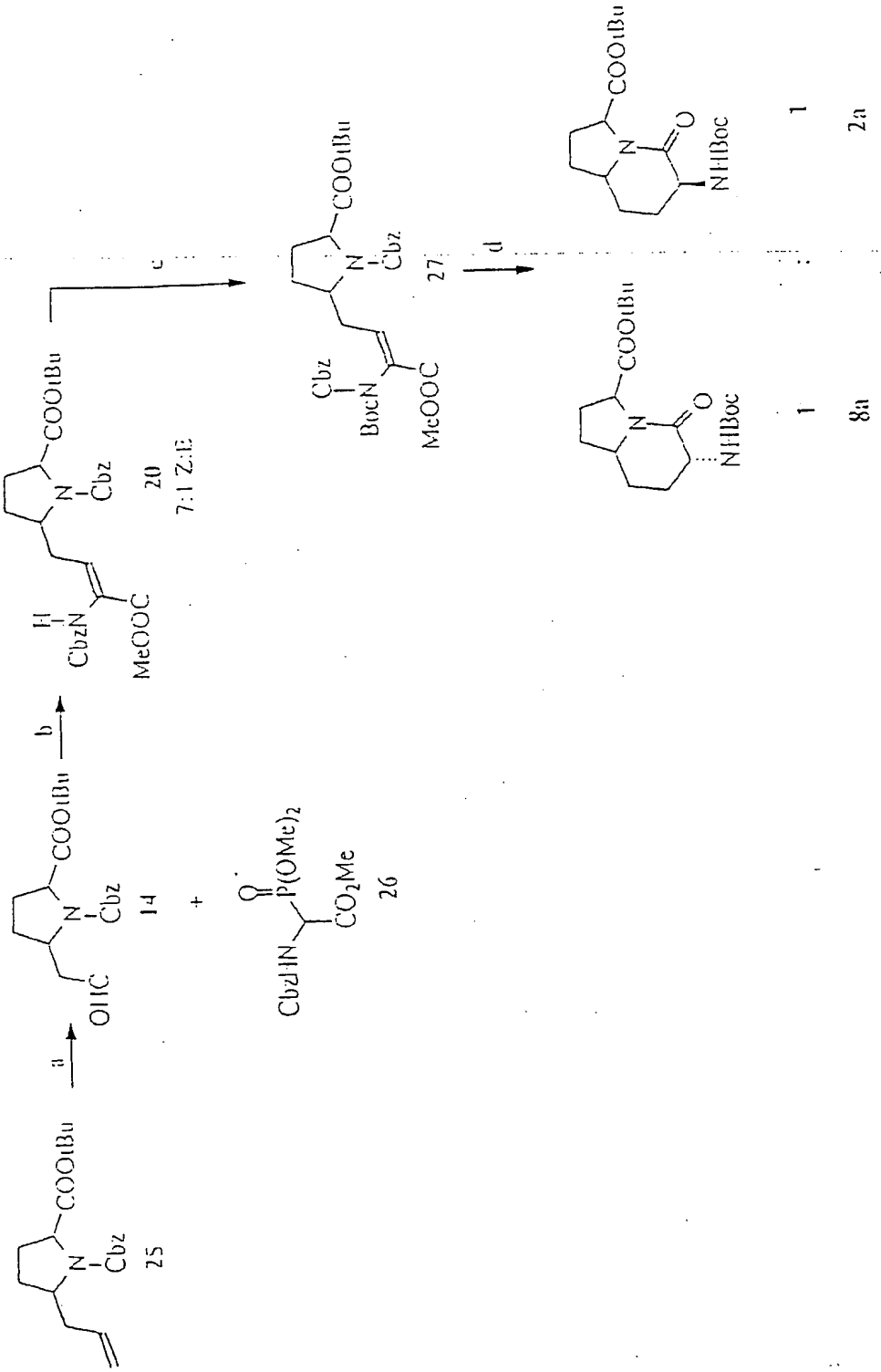

COOtBu
N
Cbz
25
a
OHC
14
b
CbzHN
P(OMe)2
O
CO2Me
26
+
CbzN
H
MeOOC
20
7:1 Z:E
c
BocN
Cbz
MeOOC
27
d
NHBoc
1
2a
1
8a

Figure 3

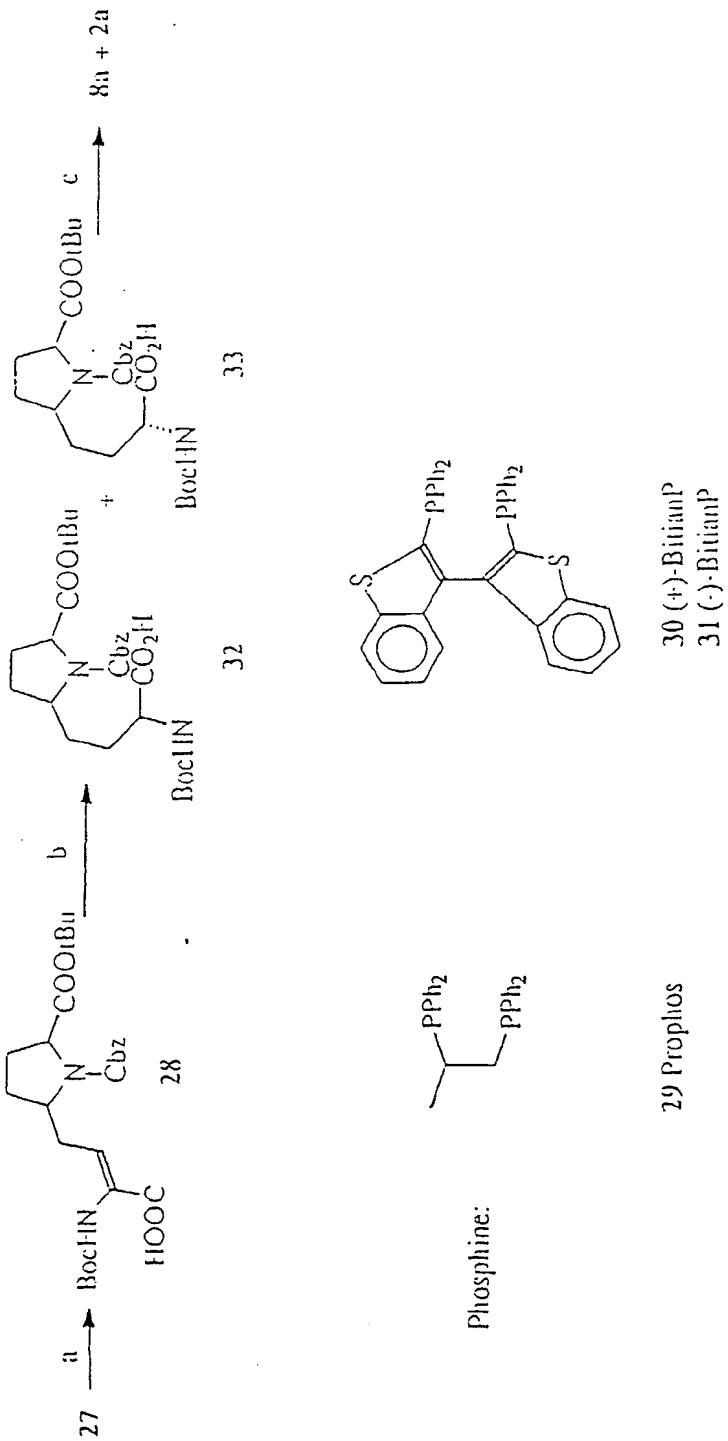


Figure 4

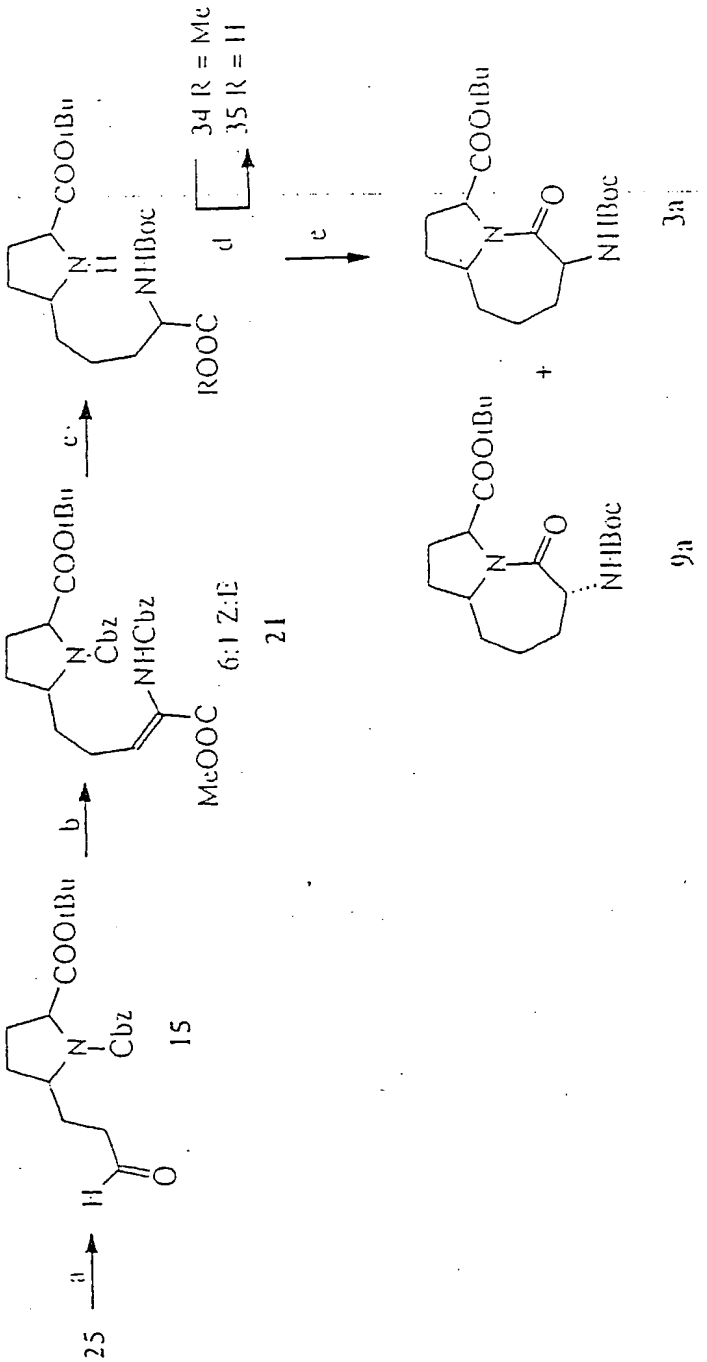

25
a
15
b
21
6:1 Z:E
c
34 R = Me
35 R = H
d
e
3a
9a
COOtBu
Cbz
NHCbz
NHBoc
MeOOC
ROOC

FIGURE 5

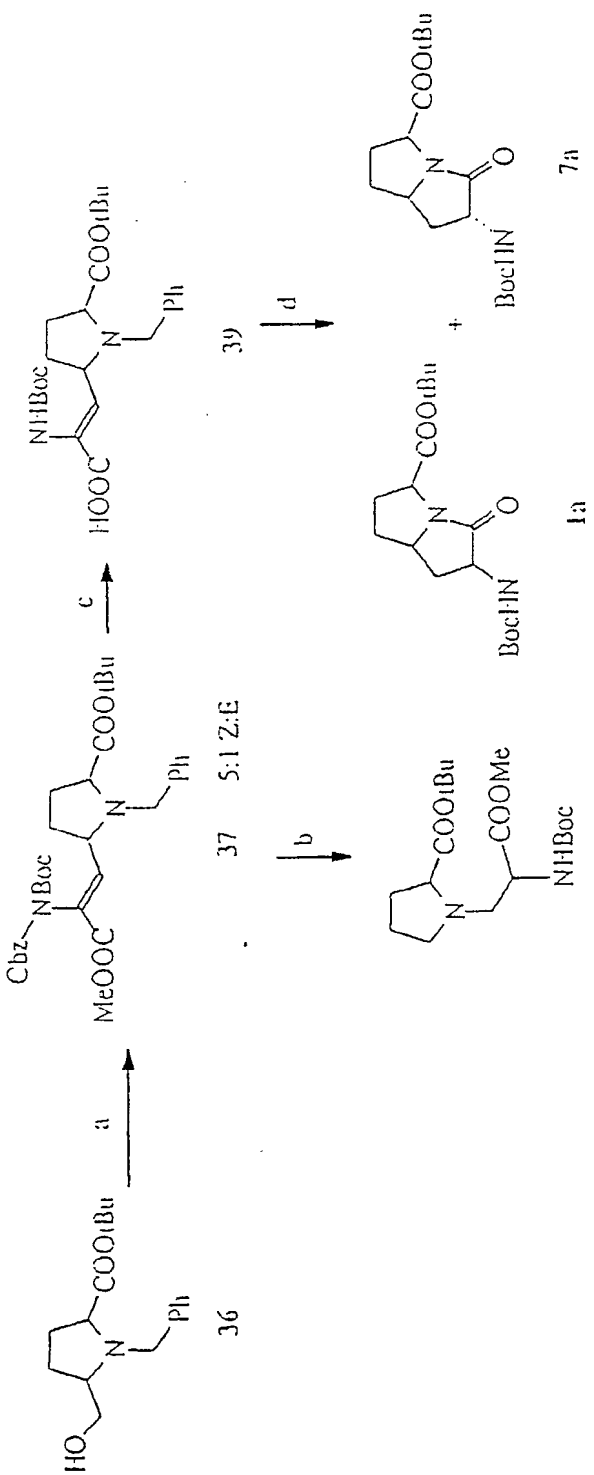


FIGURE 6

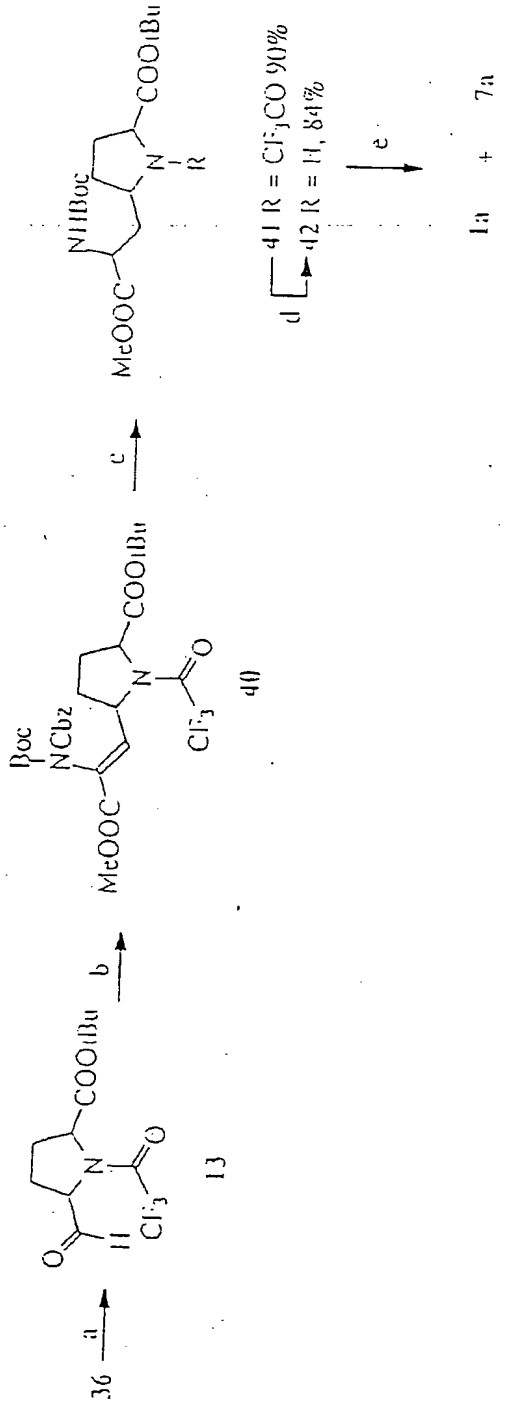


FIGURE 7

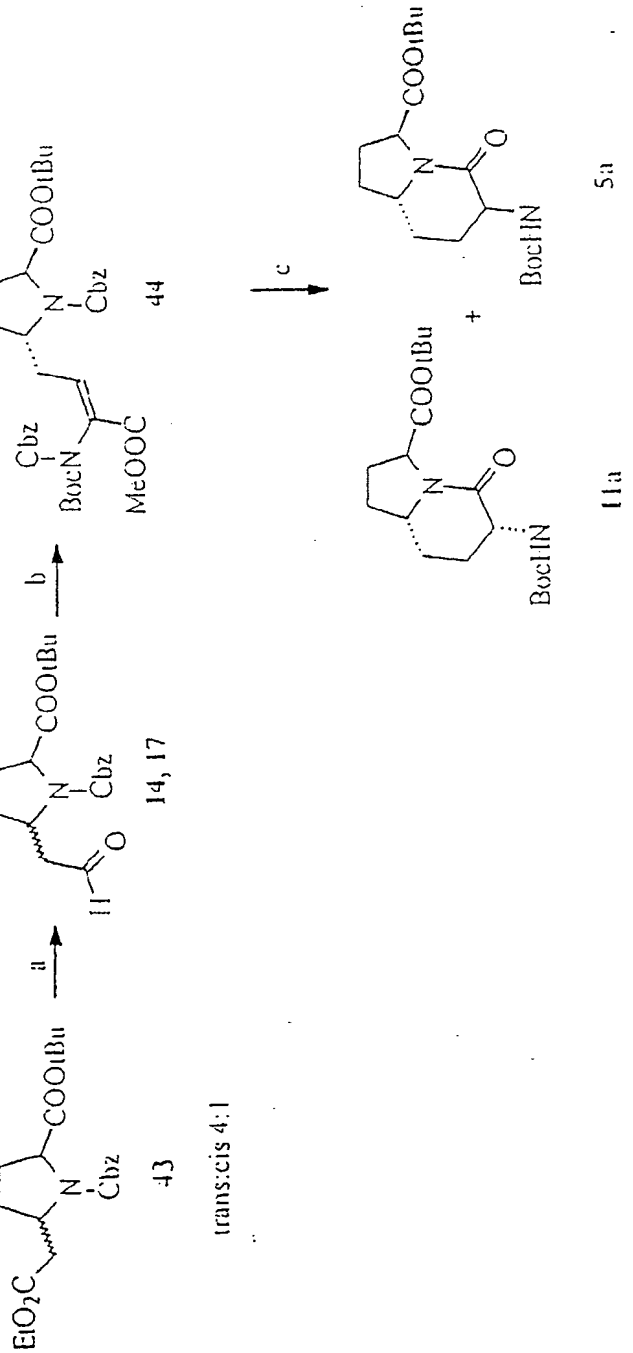


FIGURE 8

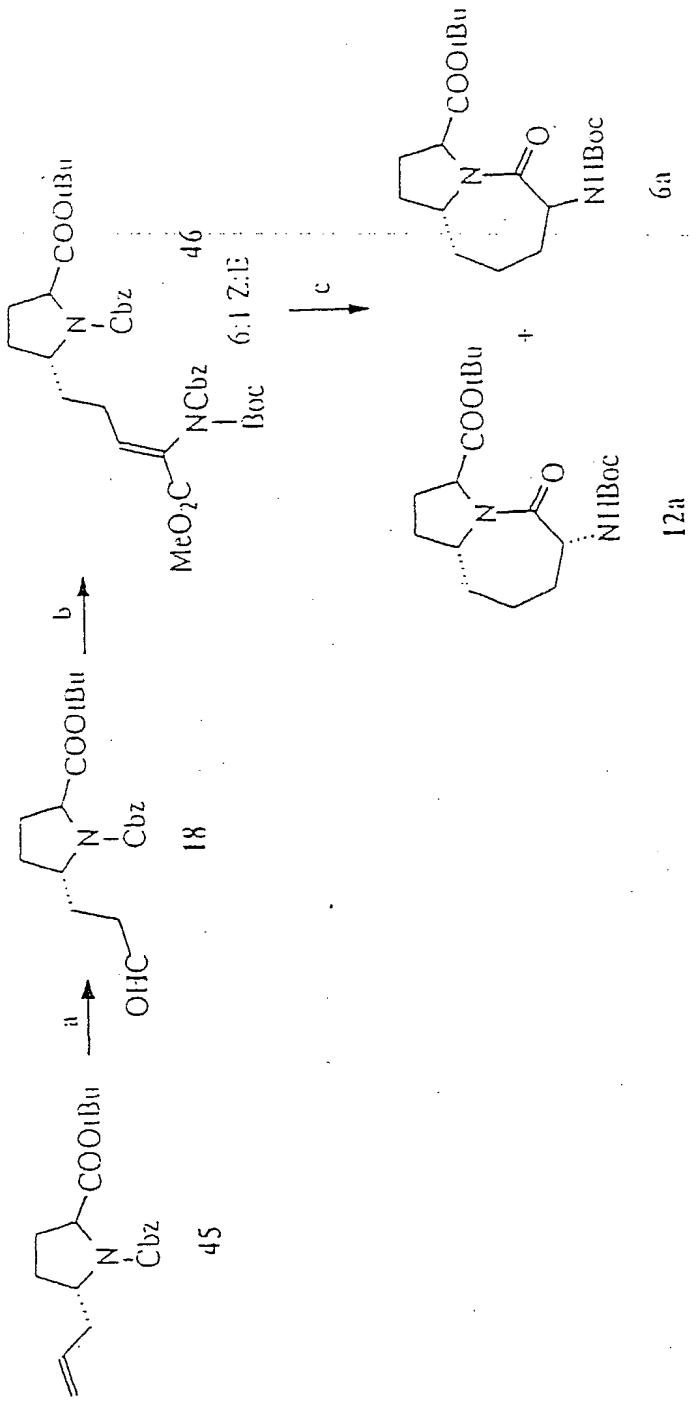
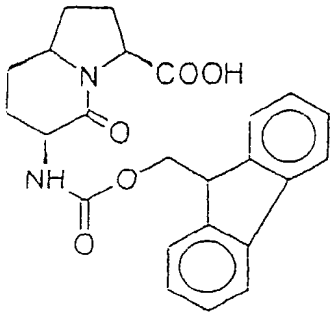
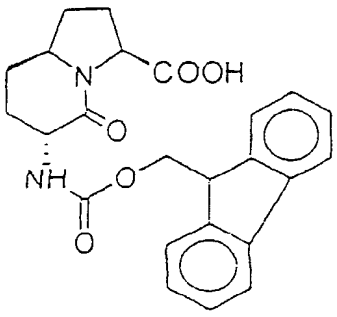


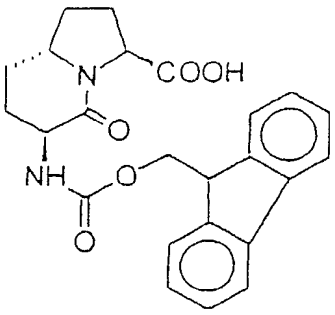
FIGURE 9



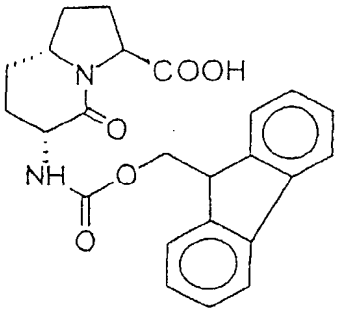
Fmoc-Temp1



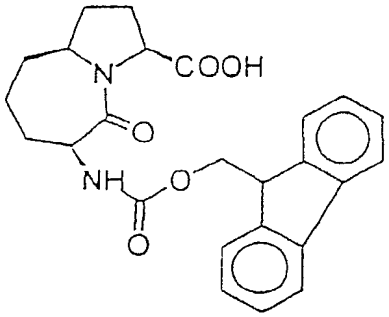
Fmoc-Temp2



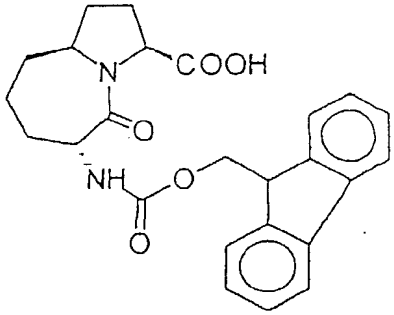
Fmoc-Temp3



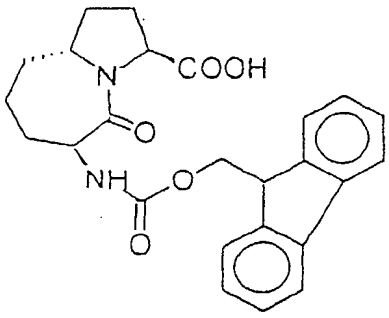
Fmoc-Temp4



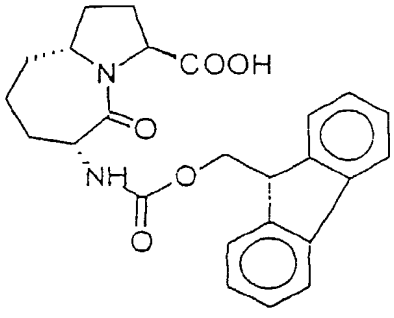
Fmoc-Temp5



Fmoc-Temp6

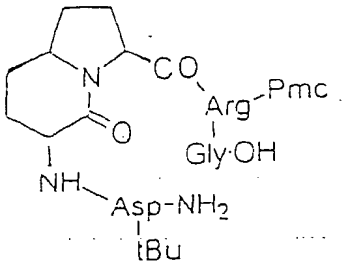


Fmoc-Temp7

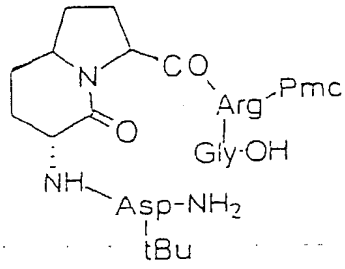


Fmoc-Temp8

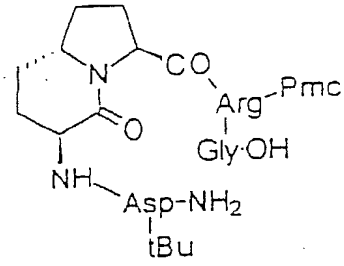
FIGURE 10



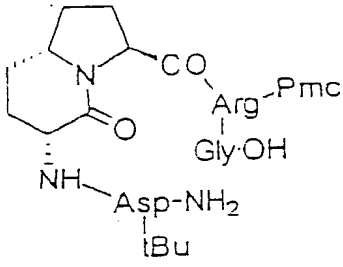
1



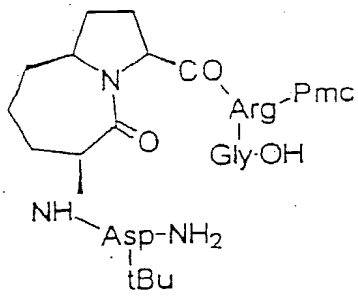
2



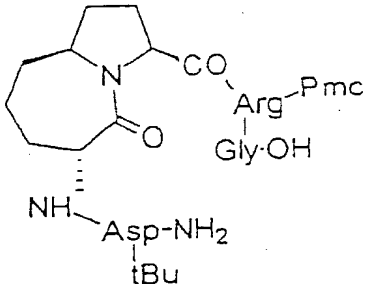
3



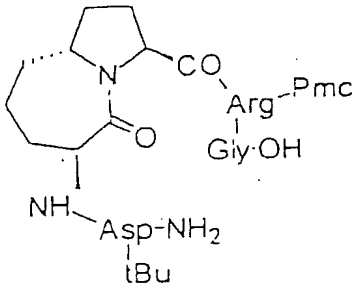
4



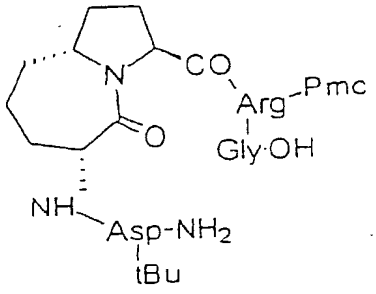
5



6

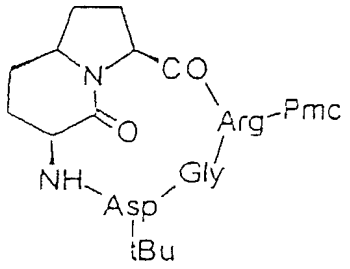


7

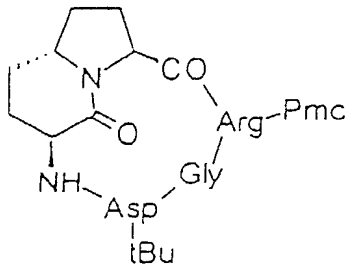


8

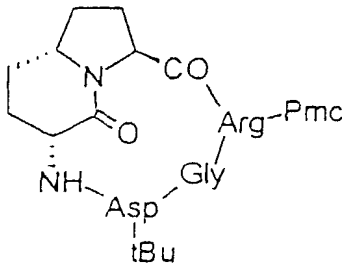
FIGURE 11



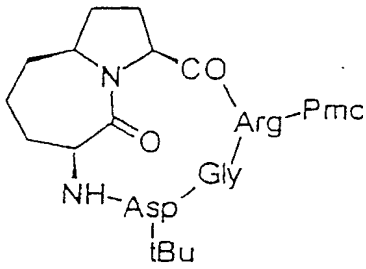
9



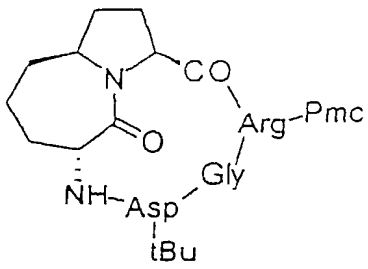
10



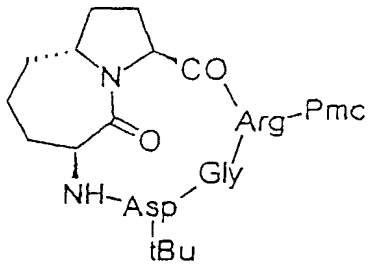
11



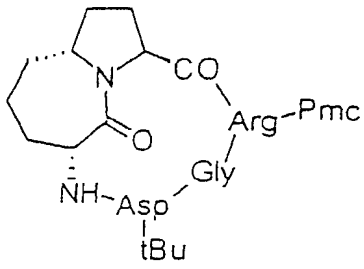
12



13

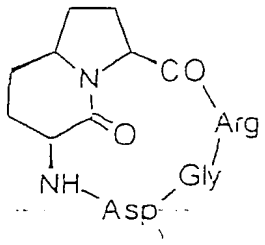


14

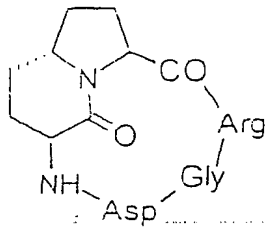


15

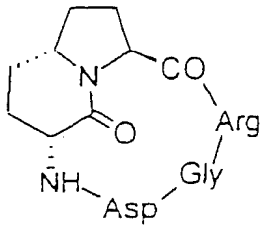
FIGURE 12



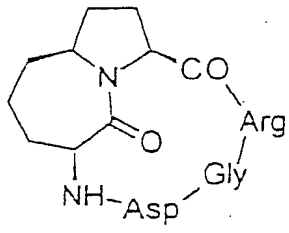
16



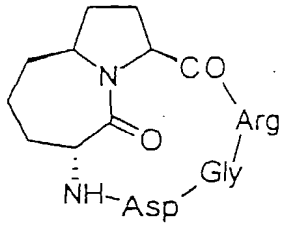
17



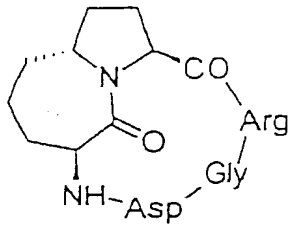
18



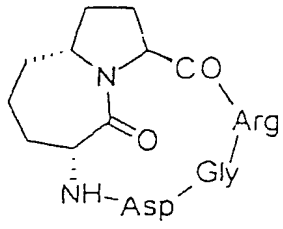
19



20



21



22

BLANK PAGE

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 077 218 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
19.12.2001 Bulletin 2001/51

(51) Int Cl.[7]: **C07K 7/56**

(43) Date of publication A2:
21.02.2001 Bulletin 2001/08

(21) Application number: **00830535.1**

(22) Date of filing: **27.07.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **04.08.1999 US 366198**

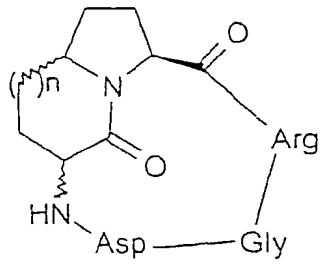
(71) Applicant: **SIGMA-TAU Industrie Farmaceutiche Riunite S.p.A.**
00144 Roma (IT)

(72) Inventors:
- **Scolastico, Carlo**
  20100 Milano Due (Segrate Milano) (IT)
- **Giannini, Giuseppe**
  00040 Pomezia, Rome (IT)

(74) Representative: **Spadaro, Marco**
Sigma-Tau Industrie Farmaceutiche Riunite SpA, 47,Viale Shakespeare
00144 Rome (IT)

(54) Peptido-mimetic compounds containing RGD sequence useful as integrin inhibitors

(57) The present invention discloses compounds of formula (I)



(I)

wherein n is the number 0, 1 or 2. There are also disclosed processes for the preparation of said compounds, together with methods for treating pathologies related to an altered $\alpha_v\beta_3$ integrin-mediated cell attachment, in particular wherein the inhibition of angiogenesis is desired, for example in tumors, also associated with metastasis.

EP 1 077 218 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 83 0535

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	WO 98 27112 A (MERCK PATENT GMBH ;GOODMAN SIMON (DE); FITTSCHEN CLAUS (DE); HOELZ) 25 June 1998 (1998-06-25) claims	1-20	C07K7/56
Y	WO 95 25543 A (SCRIPPS RESEARCH INST) 28 September 1995 (1995-09-28) Table 1, esp peptide 62187	1-20	
Y	WO 91 15515 A (JOLLA CANCER RES FOUND) 17 October 1991 (1991-10-17) whole document, esp. Table 1	1-20	
Y	WO 96 00581 A (VANDERSLICE PETER ;BECK PAMELA J (US); KOGAN TIMOTHY P (US); REN K) 11 January 1996 (1996-01-11) whole document, page 6, lines 5ff, together with claim 4	1-20	
Y	BELVISI, LAURA ET AL: "Conformational preferences of peptides containing reverse-turn mimetic bicyclic lactams. Inverse.gamma.-turns versus type-II'.beta.-turns. Insights into.beta.-hairpin stability" EUR. J. ORG. CHEM. (1999), (2), 389-400 , XP000941804 * the whole document *	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07K
A	WO 97 05160 A (PALEARI FABIO ;CRISCUOLI MARCO (IT); SALIMBENI ALDO (IT); MENARINI) 13 February 1997 (1997-02-13) claims, examples	1-20	
A	US 5 767 071 A (PALLADINO MICHAEL A ET AL) 16 June 1998 (1998-06-16) * the whole document *	1-20	

-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	26 October 2001	Kronester-Frei, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 83 0535

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	ANNIS, D. ALLEN ET AL: "Stereochemistry as a diversity element: solid-phase synthesis of cyclic RGD peptide derivatives by asymmetric catalysis" ANGEW. CHEM., INT. ED. (1998), 37(13/14), 1907-1909 , XP000941723 * the whole document *	1-20	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	26 October 2001	Kronester-Frei, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 83 0535

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

26-10-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9827112 A	25-06-1998	DE 19653036 A1	25-06-1998
		AT 199256 T	15-03-2001
		AU 722817 B2	10-08-2000
		AU 5758498 A	15-07-1998
		CN 1247543 A	15-03-2000
		CZ 9902232 A3	15-09-1999
		DE 59703023 D1	29-03-2001
		DK 948525 T3	11-06-2001
		WO 9827112 A1	25-06-1998
		EP 0948525 A1	13-10-1999
		ES 2156011 T3	01-06-2001
		HU 0000490 A2	28-08-2000
		NO 993010 A	18-08-1999
		PL 334186 A1	14-02-2000
		PT 948525 T	31-07-2001
		SI 948525 T1	31-08-2001
		SK 82099 A3	12-06-2000
		ZA 9711392 A	18-06-1999
WO 9525543 A	28-09-1995	US 5753230 A	19-05-1998
		US 5766591 A	16-06-1998
		AU 709645 B2	02-09-1999
		AU 1995295 A	09-10-1995
		CA 2184493 A1	28-09-1995
		CN 1151120 A	04-06-1997
		CZ 9602711 A3	14-01-1998
		EP 0754059 A1	22-01-1997
		FI 963692 A	18-09-1996
		HU 76086 A2	30-06-1997
		JP 10500398 T	13-01-1998
		NO 963894 A	18-11-1996
		SK 119096 A3	09-07-1997
		WO 9525543 A1	28-09-1995
		ZA 9502214 A	08-02-1996
WO 9115515 A	17-10-1991	AT 155482 T	15-08-1997
		AU 3424695 A	14-03-1996
		AU 660926 B2	13-07-1995
		AU 7763191 A	30-10-1991
		CA 2079606 A1	07-10-1991
		DE 69126871 D1	21-08-1997
		DE 69126871 T2	12-03-1998
		DK 527798 T3	15-12-1997
		EP 0527798 A1	24-02-1993
		EP 0749979 A2	27-12-1996
		ES 2104702 T3	16-10-1997

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 83 0535

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

26-10-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9115515 A		FI 924472 A	05-10-1992
		GR 3024996 T3	30-01-1998
		JP 5509294 T	22-12-1993
		NO 923875 A	04-12-1992
		WO 9115515 A1	17-10-1991
		US 6017877 A	25-01-2000
		US 6013625 A	11-01-2000
		US 5612311 A	18-03-1997
		US 5672585 A	30-09-1997
		US 5648330 A	15-07-1997
		US 5780303 A	14-07-1998
		US 6100236 A	08-08-2000
WO 9600581 A	11-01-1996	AU 2958195 A	25-01-1996
		CA 2193828 A1	11-01-1996
		EP 0767674 A1	16-04-1997
		JP 10502349 T	03-03-1998
		US 6087330 A	11-07-2000
		WO 9600581 A1	11-01-1996
WO 9705160 A	13-02-1997	IT MI951688 A1	03-02-1997
		AU 6734296 A	26-02-1997
		WO 9705160 A1	13-02-1997
US 5767071 A	16-06-1998	NONE	

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

BLANK PAGE

BLANK PAGE